No. 6. The Examiner then stated that the originally presented claims have received an action on the merits and therefore have been constructively elected. As a consequence, Claims 109-160 were withdrawn from consideration.

The restriction requirement is traversed.

On page 2, line 5-6, the Office Action states "newly submitted Claim 109-160 are independent and distinct from the invention originally claimed for the following reasons: The compound and the method of use is distinct and independent as evident from the instant claims and would have been subject to restriction requirement made in the paper # 6". However, ***no reasons are provided***; the Examiner has merely asserted that Claims 109-160 are independent and distinct from the other claims. According to MPEP 803 Examiners must provide reasons to support a restriction:

> Examiners must provide reasons and/or examples to support conclusions, but need not cite documents to support the restriction requirement in most cases.

Unless a proper justification for the restriction requirement is provided, it should be withdrawn.

It is noted that the subject matter of Claims 109-122 is completely encompassed within Claim 15; and the subject matter of Claims 123-160 is completely encompassed within Claim 71. Therefore, the Examiner has already searched the subject matter of new Claims 109-160 and there is no additional search burden on the Examiner in examining new Claims 109-160. The MPEP stipulates that claims directed to inventions that are otherwise independent and distinct should be examined when there is no additional search burden on the examiner[1]:

> If the search and examination of an entire application can be made without serious burden, the examiner must examine it on the merits, even though it includes claims to independent or distinct inventions. (MPEP 803).

For this reason, Applicants are requesting withdrawal of the restriction requirement.

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[1] Applicants are not acknowledging that Claims 109-160 are directed to an invention independent and distinct from the subject matter of the originally presented claims.

The restriction requirement should be withdrawn for yet other reasons. Because Claims 15 and 71 are generic to new Claim 109-160, they are properly classified as "linking claims":

> There are a number of situations which arise in which an application has claims to two or more properly divisible inventions, so that a requirement to restrict the application to one would be proper, but presented in the same case are one or more claims (generally called "linking" claims) inseparable therefrom and thus linking together the inventions otherwise divisible. The most common types of linking claims which, if allowed, **act to prevent restriction** between inventions that can otherwise be shown to be divisible, are (A) **genus claims linking species claims**. (MPEP 809.03) (Emphasis added).

Claims 15 and 71 are inseparable from the subject matter of new Claims 109-160 because they are drawn to the same subject matter (a compound which modulates TNF-α activity and methods of using the same in therapy) and because Claims 15 and 71 are generic to new Claims 109-160. Thus, Claims 15 and 71 ***link*** Claims 109-160 and ***act to prevent restriction*** between them. Therefore, the second restriction is improper and should be withdrawn.

In summation, the Examiner has not provided a rationale justifying the restriction requirement. Moreover, the restricted Claims (109-160) are completely encompassed within old Claims 15 and 71; thus, there will no additional search burden on the Examiner in examining these claims. Finally, restriction is improper because Claims 15 and 71 are generic to new Claims 109-160 and therefore serve to "link" them and prevent restriction. Withdrawal of the restriction requirement is requested.

Rejection Under 35 U.S.C. § 112, Second Paragraph

Claims 79 was rejected because it reads "the compound of Claim 71"; however, Claim 71 is not directed to a compound. This informality has been corrected by amendment. Withdrawal of the rejection is requested.

Rejection of Claim 71-100 and 108 Under 35 U.S.C. § 112, First Paragraph

The Examiner stated that the specification is enabling for the treatment of rheumatoid arthritis. However, the Examiner stated that Claims 71-100 and 108 are drawn to treating "TNF-mediated conditions" and therefore "not only include any and all conditions, but also those conditions not yet to be discovered for which there is no enabling disclosure".

Applicants acknowledge the Examiner's assessment that the specification is enabling for the treatment of rheumatoid arthritis, but, for reasons provided below, respectfully disagree with his conclusions that the claims lack enablement for other TNF-mediated diseases.

A. The Examiner has provided no rationale to question enablement of the claimed subject matter

An examiner may not simply assert lack of enablement, but must provide reasons to questions the validity of what is asserted. As stated in MPEP 2164.04:

> As stated by the court, "it is incumbent upon the Patent Office, whenever a rejection on this basis is made, to explain why it doubts the truth or accuracy of any statement in a supporting disclosure and to back up assertions of its own with acceptable evidence or reasoning which is inconsistent with the contested statement. Otherwise, there would be no need for the applicant to go to the trouble and expense of supporting his presumptively accurate disclosure." 439 F.2d at 224, 169 USPQ at 370.
>
> According to In re Bowen, 492 F.2d 859, 862-63, 181 USPQ 48, 51 (CCPA 1974), the minimal requirement is for the examiner to give reasons for the uncertainty of the enablement.

The Examiner has provided no such rationale, other than to state in Section 2 on page 5 of the Office Action that "[a] very recent publication expressed that treating diseases by the inhibition of TNF-α is still exploratory. See reference cited above." However, Applicants' Attorney has not been able to identify any reference cited in the Office Action which makes this assertion. In absence of such documentation, the Examiner's assertion of lack of enablement lacks the support required by MPEP 2164.04.

B. Clinical data is not a requirement for patentability

Example 17 of the subject application discloses data from two *in vitro* assays: the first measures TNF-α induced apoptosis; and the second measures Vascular Cell Adhesion Molecule expression. Both assays measure outcomes from TNF-α treatment, and, as such, are valid assays for identifying molecules which inhibit TNF-α signalling. ***Ninety-two*** compounds encompassed within Claim 75 were tested and found to be active by these assays. Moreover, Compound **44**

was tested and found to be active in animal models for sepsis (Example 18 beginning on page 77), inflammatory bowel disease (IBD) (Example 18 beginning on page 77) and allergic encephalitis (encephalomyelitis) (Example 18, beginning on page 78), which is a murine model of multiple sclerosis. Thus, it is apparent that the disclosed assays provide at least a reasonable correlation between inhibition of TNF-α signalling *in vitro* and efficacy in animal models. Based on this data, it is reasonable to conclude that the disclosed inhibitors of TNF-α signalling would also be effective against other TNF-mediated disorders, and at least against sepsis, IBD, allergic encephalitis (encephalomyelitis) and multiple sclerosis.

The Examiner stated in Section 4 on page 5 of the Office Action that the "[s]pecification has no working examples to show treating any or all condition". Based on the assays disclosed in the subject application, as described in the previous paragraph, and the number of examples tested, Applicants respectfully disagree with this assertion. Perhaps the Examiner is suggesting that clinical data is required for patentability. If so, this is not the law. The Court of Appeals for the Federal Circuit has repeatedly admonished that clinical data is not a requirement for patentability. See, for example, *Cross v. Iizuka*, 224 USPQ 739 (CAFC, 1985):

> We perceive no insurmountable difficulty under appropriate circumstances, in finding that the first link in the screening chain, in vitro testing, may establish a practical utility for the compound in question. *Cross* at 748.
>
> Since we have agreed with the Board that the practical utility for the imidazole derivatives of the phantom count lies in their pharmacological activity in the microsome environment, the how to use requirement of §112 must be analyzed with reference to the microsome environment. *Cross* at 748.

See, also, *In re Brana* 34 USPQ2d 1437, 1442 (CAFC 1995).

> FDA approval, however, is not a prerequisite for finding a compound useful within the meaning of the patent laws. *Scott,* 34 F.3s 1058, 1063, 32 USPQ2d 1115, 1120. Usefulness in patent law, and, in particular in the context of pharmaceutical inventions, necessarily includes the expectation of further research and development. The stage at which an invention in this field becomes useful is well before it is ready to be administered to humans. *In re Brana* at 1442.

Thus, clinical efficacy is not a requirement for patentability. The specification provides ninety-two examples of *in vitro* efficacy and further data showing efficacy in animal models with at

least three different TNF-α mediated diseases. Based on this data, it is reasonable to conclude efficacy against other TNF-mediated disorders.

C. The use of TNF-α inhibitors is well known for treating a wide variety of inflammatory and autoimmune disorders.

The Examiner's assertion that "the state of the art is that the effects of inhibiting TNF-α are unpredictable and at best limited to modulation of rheumatoid arthritis" is incorrect. For example, Infliximab, a monoclonal antibody against TNF-α, has obtained FDA approval for the treatment of Crohn's disease (Andreakos *et al., Cytokine and Growth factor Review 13:*299 (2002) [enclosed herewith as Exhibit A] and Present *et al.,. N. Engl. J. Med. 340:*1398 (1999) [enclosed herewith as Exhibit B]; and etanercept improved clinical disease in 74% of the patients with juvenile arthritis compared to placebo (Lovell *et al., N. Engl. J. Med. 342:*763 (2000)) [enclosed herewith as Exhibit C]. Moreover, clinical trials have demonstrated the efficacy of TNF-α inhibitors for treating psoriatic arthritis and psoriasis (Mease *et al., Lancet 356:*9227 (2000)) [Exhibit D], Wegener's granulomatosis (Stone *et al., Arthritis Rheum. 43(Suppl):*S404 (2000)) [Exhibit E], adult onset Still's disease (Weinblatt *et al., Arthritis Rheum. 43(Suppl):*S391 (2000)) [Exhibit F], polymyositis (Hengstman *et al., Arthritis Rheum 43(Suppl):*S193 (2000)) [Exhibit G] and scleroderma (Ellman *et al., Arthritis Rheum. 43(Suppl):*S392 (2000)) [Exhibit H]. In addition, clinical efficacy in mice with encephalomyelitis (EAE), a relevant model for human demyelinatin disease such as multiple sclerosis, has been demonstrated with TNF-α inhibitors (Lock *et al., Ann. Rheu. Dis. 58(Suppl I):*I121 (1999) [Exhibit I]. These references have been enclosed as Exhibits for the convenience of the Examiner with relevant portions highlighted.

Thus, clinical efficacy for TNF-α inhibitors has been demonstrated in a wide variety of diseases other than rheumatoid arthritis. It is therefore reasonable to expect efficacy for inhibitors of TNF-α signalling in these and other TNF-α mediated disorders.

In summation, the clinical efficacy of TNF-α inhibitors against a wide variety of TNF-α mediated disorders has been established and is well known. It is to be expected that inhibitors of TNF-α signaling will be useful in treating these and other TNF-α mediated diseases. The subject

signalling. Efficacy in three different animal models is also demonstrated. From this data and the widespread utility of TNF-α inhibitors against TNF-α mediated diseases, it is submitted that the full scope of Claims 71-100 and 108 has been enabled.

Rejection Under 35 U.S.C. § 103(a)

Claims 15-16, 20-23, 26-27 and 29 are rejected as being obvious in view of U.S. Patent No. 6,306,840 (hereinafter "Adams"). The Examiner stated that Adams teaches compounds structurally similar to those claimed by Applicants. The Examiner also stated that the claimed compounds differ from those disclosed in Adams in requiring (un)substituted aryl for R_{10}, whereas Adams exemplifies only hydrogen and methyl for the corresponding R_2. The Examiner then stated that Adams teaches the equivalency of exemplified substituents shown in Table I with that claimed for compounds of formula (I) in column 6. For reasons provided below, Applicants respectfully disagree with this assessment.

The Examiner noted that R_2 in formula (I) in column 6 of Adams corresponds to R_{10} in the instant claims. However, only ***two out of eight*** recited values for R_2 in formula (I) in column 6 of Adams are permissible values for R_{10} in the instant claims. Moreover, R_3 in formula (I) in column 6 of Adams corresponds to R_9 in the instant claims, and only ***five out of approximately 35*** recited values for R_3 in formula (I) in column 6 of Adams are permissible values for R_{10} in the instant claims. In addition, Y-R1 in formula (I) in column 6 of Adams corresponds to -C(O)-R_{11} in the instant claims, and only ***one out of the three*** recited values for Y in formula (I) in column 6 of Adams results in a permissible value for -C(O)-R_{11} in the instant claims. Adams ***provides no reason to select these values.*** It is well established in Patent Law that an obviousness rejection is improper if the skilled person must make a specific selection from a large number of variables, each having a large number of possible values, unless the prior art directs the skilled person to make the specific selections necessary to arrive at the claimed subject matter:

> Given the vast number of diphenols encompassed by the generic diphenol formula in Knapp, and the fact that the diphenols that Knapp specifically discloses to be "typical," "preferred," and "optimum" are different from and more complex than bisphenol A, we conclude that Knapp does not teach or farily suggest the selection of bisphnol A. *In re Baird* 29 USPQ2d 1150, 1552 (CAFC 1994).

Not only does Adams fail to direct the skilled person to the specific selections of R_2, R_3 and Y necessary to arrive at permissible values for R_9, R_{10} and $-C(O)R_{11}$ in the instant claims, but it actually teaches away from these selections. Table 1, columns 9-18 in Adams provides almost four hundred specific examples of compounds encompassed by formula (I) in column 6. Values for R_2 are limited to -H and -methyl (and in one case phenacyl); in contrast, R_{10}, which is at the corresponding position in the compound of Applicant's Claim 15, is required to be alkyl substituted with an amine or a substituted or unsubstituted aryl, heteroaralkyl or heterocycloalkylalkyl. In addition, of the almost four hundred examples in Table 1, only approximately nine have a substituted or unsubstituted aryl, heteroaryl, aralkyl or heteroaralkyl group as a possible for R_3, as required by R_9, the variable at the corresponding position in the compound of Claim 15. As such, none of the specific examples disclosed in Adams directs the skilled person to select the permitted values for R_{10} of the compound of Claim 15, and only a tiny percentage have a permissible value for R_9 of the compound of Claim 15. None suggest the specific combination of required values.

Compounds encompassed within the generic structure of Claim 15 are non-obvious for yet other reasons. Specifically, the activity of these compounds is unexpectedly superior to the corresponding compounds in which the group represented by R_{10} is -H or alkyl. It is noted that the examples provided in Table 1 of Adams have -H or methyl (and in one case phenacyl) at the position corresponding to R_{10}. Support for this assertion is provided by the enclosed Declaration by Scott F. Sneddon, Ph.D. (hereinafter the "Sneddon Declaration"). The Sneddon Declaration provides results from experiments in which Compounds **1-4** were tested in the TNF-α induced apoptosis assay described on pages 70-71 of the subject application and the IC_{50}s were determined. The group at the position represented by R_{10} in Compounds **1-4** is -H or alkyl. In every case, the activity of these compound in the TNF-α induced apoptosis assay is greater than 30 μM; in contrast, the activity of the compounds of the present invention is in every case less than 10 μM, and in many cases less than 1 μM (see Table V on pages 73-75 of the subject application). Thus, it is apparent that the claimed compounds have superior activity to the corresponding compounds that have -H or alkyl at the position corresponding to R_{10}, as in the compounds in Table 1 of Adams.

In summation, the subject matter of Claim 15 and claims depending therefrom is non-obvious in view of Adams because it is necessary to select specific values from a large number of possible values of R_2, R_3 and Y in order to arrive at the permissible values for R_9, R_{10} and $-C(O)-R_{11}$ in the compound of Claim 15. Moreover, Adams teaches away from these specific selections because none of Adams exemplified compounds have these selections. Finally, compounds with values for R_9, R_{10} and $-C(O)R_{11}$ that are permitted by Claim 15 are unexpectedly superior in their ability to inhibit TNF-α, compared with the corresponding compounds having -H or alkyl at R_{10}. It is noted that all of the examples provided by Adams have -H or alkyl (and in one case phenacyl) at the position corresponding to R_{10}.

CONCLUSION

In view of the above amendments and remarks, it is believed that all claims are in condition for allowance, and it is respectfully requested that the application be passed to issue. If the Examiner feels that a telephone conference would expedite prosecution of this case, the Examiner is invited to call the undersigned at (978) 341-0036.

Respectfully submitted,

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Dated: July 9, 2003

MARKED UP VERSION OF AMENDMENTS

Claim Amendments Under 37 C.F.R. § 1.121(c)(1)(ii)

79. (Amended) The [compound] method of Claim 71, wherein R_9 is phenyl, 2-cyanophenyl, 3-cyanophenyl, 4-cyanophenyl, diphenylmethyl, pyrazolylmethyl, 2,4-dimethylphenyl, 2-methylphenyl, 3-methylphenyl, 4-methylphenyl, 2-methyl-4-methoxyphenyl, 3-methyl-4-methoxyphenyl, 4-methylthiophenyl, 3-chlorophenyl, 3-trifluoromethylphenyl, benzyl, 2-trifluoromethylbenzyl, 3-trifluoromethylbenzyl, 2-chlorobenzyl, 3-chlorobenzyl, 4-chlorobenzyl, 2-methoxybenzyl, 3-methoxybenzyl, 4-methoxybenzyl, 2-fluorobenzyl, 3-fluorobenzyl, 4-fluorobenzyl, 3-azidylphenyl, 3-(4-methoxyphenoxy)phenyl, or 5-phenylfuran-2-yl.

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Survey

Cytokines and anti-cytokine biologicals in autoimmunity: present and future

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Abstract

The increasing understanding of the role of cytokines in autoimmunity, and the observation that tumour necrosis factor α (TNFα) is central to the inflammatory and destructive process common to several human autoimmune diseases, has led to a new generation of therapeutics, the TNFα blocking agents. In this article, we review the current knowledge of the role of cytokines in autoimmunity as unravelled by studies both in the laboratory and the clinic. In addition, we discuss future prospects of the anti-TNFα therapy that may involve combination therapy with other anti-cytokine or anti-T cell biologicals, or the use of small chemicals targeting molecules involved in TNFα production such as NF-κB and p38 MAPK. The future developments of anti-TNFα and anti-cytokine therapy in general will be interesting.

Keywords: Autoimmunity; Rheumatoid arthritis; Cytokine; TNF; NF-κB

Contents

1. Introduction

Autoimmune diseases involve the activation of self-reactive T and B cells and the generation of cell-mediated and humoral immune responses against self-antigens. A large number of autoimmune diseases have been described, with the most common of them being rheumatoid arthritis (RA), multiple sclerosis (MS), systemic lupus erythematosus, insulin-dependent diabetes mellitus, Grave's and Hashimoto's thyroiditis, ankylosing spondylitis and Crohn's disease. Although clinically distinct, all autoimmune diseases have some similarities in their pathogenesis and involve the production of cytokines, important protein mediators that specifically regulate the inflammatory response, the tissue damage and the repair mechanisms [1]. In this article, we will review the role of cytokines in the pathogenesis of human autoimmune diseases, and discuss

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how a partial understanding of how the cytokine networks operate has led to new therapies.

2. Initiation, perpetuation and tissue damage in autoimmune disease

The events that trigger autoimmunity or that determine the disease type, manifestation and progression remain elusive. Certainly, the strong association of autoimmune diseases with particular MHC genotypes, and also with a number of other immune and inflammatory genes or with the hormonal status of the individual indicates that genetic factors are important [2–4]. However, autoimmune diseases are clinically manifested in <50% of identical twins that share all their genes, e.g. the concordance rates of identical twins in RA is 15–35%. This demonstrates that non-inherited and presumably environmental factors are also involved [2,5].

No matter how autoimmunity is initiated, it results in a chronic inflammatory response against self-tissue with the release of inflammatory mediators, the production of autoantibodies, the formation of immune complexes and the extravasation and activation of cytotoxic T cells, natural killer cells, macrophages and polymorphonuclear leukocytes. Autoantigen-specific antibodies induce tissue damage by either directly lysing cells, accelerating clearance of antibody-sensitised cells, activating complement, initiating antibody-dependent cytotoxicity mechanisms or by binding to cell surface receptors and interfering with their function. T cells, on the other hand, mediate tissue injury directly by themselves by releasing cytokines and attacking self-tissue, or indirectly by providing help to autoantigen-specific B cells and by activating macrophages and inducing local inflammation. Such mechanisms seem to operate in rheumatoid arthritis and multiple sclerosis that have disease tissues heavily infiltrated by T cells and activated macrophages [6]. Ultimately, this process results in extensive tissue damage and leads to malfunction of the corresponding target organs, disability or even death.

Extensive damage of the tissues is characteristic of the late or tissue-damaging stage of autoimmunity, and is often already taking place when the disease is diagnosed. This is the stage of autoimmunity that is the most relevant therapeutically, but also the most accessible for study. The earlier stages of autoimmunity are envisaged to contain an 'initiation' stage that is largely asymptomatic and involves the induction of autoimmunity presumably under the influence of both genetic and environmental factors, and a 'perpetuation' stage that results in the persistence of autoreactive T cells and the maintenance of inflammation. There is increasing evidence that all stages of autoimmunity are controlled by cytokines and their study has been a major subject of the research of the last 15 years in the field. Most of the data concerning early events come from animal models, but there are many grounds to believe their relevance to human disease [7,8].

3. The cytokine system in rheumatoid arthritis

One of the best studied cytokine networks of human autoimmune diseases is that of rheumatoid arthritis. Its analysis has been favoured by the availability of biopsy tissue from the disease site that can be obtained at the height of the inflammatory response [9]. Biopsy tissue can then be analysed for the expression of cytokines by various methods that include immunohistology of fresh ex vivo tissue [10], in situ hybridisation of synovial fluid products [11], and short-term culture of synovial membrane cells in the absence of extrinsic stimulation [12]. Although immunohistology is very useful when determining which cells are the major cytokine producers, the short-term culture of synovial cells offers the advantage of allowing the quantitative analysis of proteins released and the study of their regulation, and is the approach we most extensively used. However, conclusions from such in vitro studies, which have potential artefacts, require subsequent validation in vivo.

Studies evaluating the expression of cytokines in the rheumatoid synovium were initiated in the 1980s, when cytokine cDNAs were cloned and tools to measure cytokine expression became available. Very early, we and others in the field realised from the results that the rheumatoid synovium is enriched with almost every cytokine known (Table 1) [13].

The abundance of most cytokines in the rheumatoid synovium did not come as a surprise as this site is very inflammed and contains a diverse spectrum of cells such as activated T lymphocytes, macrophages, fibroblasts, endothelial cells and plasma cells, all of which can produce cytokines. However, it highlighted the complexity of determining which cytokines may be important or rate-limiting for the pathogenesis of the disease. As different cytokines exert multiple functions and have various properties, and as most cytokines are expressed transiently and can be induced or inhibited by other cytokines, we envisaged that a cytokine 'network' or 'cascade' may exist in the diseased tissue. We were struck by the reproducible presence of many key pro-inflammatory molecules in our samples, including interleukin-1 (IL-1), IL-6 and tumour necrosis factor α (TNFα), cytokines transiently produced after stimulation. This suggested to us that there may be an abnormality in the duration of cytokine expression.

To understand the way cytokines are regulated, we decided to use short-term cultures of all the cells in rheumatoid synovial membranes as an in vitro model of the cytokine network that operates in vivo. Short-term (5–6 days) cultures of rheumatoid synovial membranes contain 30% T cells, 30–40% macrophages, and fewer fibroblasts, dendritic cells, endothelial cells, plasma cells and B lymphocytes. These cells reagreggate in vitro, and spontaneously release the pro-inflammatory mediators that are also produced in vivo [9]. Using these short-term cultures and neutralising antibodies against specific cytokines as a tool for investigating regulation, we made some very interesting observations.

Table 1
Cytokines expressed in rheumatoid synovial tissue (modified with permission from Feldmann et al. [9])

Cytokines	Expression	
	mRNA	Protein
Pro-inflammatory		
IL-1α,β	+	+
TNFα	+	+
LT	+	±
IL-6	+	+
GM-CSF	+	+
M-CSF	+	+
LIF	+	+
Oncostatin M	+	+
IL-2	+	±
IL-3	−	−
IL-7	?	?
IL-9	?	?
IL-12	+	+
W-15	+	+
IFNc1α/β	+	+
IFNγ	+	±
IL-17	+	+
IL-18	+	+
Immunoregulatory		
IL-4	±	−
IL-10	+	+
IL-11	+	+
W-13	+	+
TGFβ	+	+
Chemokines		
IL-8	+	+
Groα	+	+
MIP-1	+	+
MCP-1	+	+
ENA-78	+	+
RANTES	+	+
Growth factors		
FGF	+	+
PDGF	+	+
VEGF	+	+

First, we found that by blocking TNFα but not the closely related LTα (that also binds to the same p55 TNF receptor (p55-TNFR) and p75 TNF receptor (p75-TNFR)) reproducibly inhibited the production of IL-1 [14], a cytokine previously shown to be important in cartilage and bone destruction [15,16]. This was an important observation. Although IL-1 can be induced by many molecular signals such as IL-1 itself [17], GM-CSF [18], IFNγ [19] or immune complexes [20], but also cellular interactions in the rheumatoid synovium [21], its expression is dependent on TNFα. Subsequently, we found that blocking TNFα also inhibited the production of other pro-inflammatory mediators, such as GM-CSF, IL-6 and IL-8 [22,23]. These observations were consistent with those of others [24] and indicated that many of the major pro-inflammatory cytokines produced in the rheumatoid synovium are linked in a network or cascade with TNFα at its apex. The direct implication of these findings was that blocking TNFα could be a good therapeutic approach. This hypothesis was then tested and validated by us and others in murine models of arthritis such as collagen-induced arthritis that confirmed the importance of TNFα in the induction and maintenance of disease [25–28]. Interestingly, at that time a 'spontaneous' model of arthritis was developed using a deregulated TNFα transgene carrying a deletion in the 3′ untranslated region of TNFα that is essential for its normal regulation [29]. This developed an erosive disease with histological features closely resembling human RA. Disease in this model could be prevented by using anti-human TNFα but also by a neutralising antibody to the IL-1 receptor, thus, confirming that TNFα is important in the generation of IL-1 in vivo [30]. Fong et al. also showed that in mice injected with LPS, IL-1 and IL-6 production was markedly reduced in the presence of anti-TNFα [31]. Thus, a TNFα-dependent cytokine cascade is not a feature restricted to rheumatoid synovium; rather, it is a feature of normal immune and inflammatory responses.

In addition to the pro-inflammatory mediators, the rheumatoid synovium was found to have up-regulated a number of anti-inflammatory mediators that include IL-10, IL-11, IL-1 receptor antagonist (IL-1ra) and soluble TNF or IL-1 receptors. These anti-inflammatory cytokines are effective in vivo, and partially down-regulate the inflammatory response. Thus, blocking IL-10 in rheumatoid synovial membrane cultures by anti-IL-10 neutralising antibodies increases the spontaneous production of both TNFα and IL-1, as well as IFNγ, a cytokine that is barely detectable otherwise [32]. This is in agreement with studies in human monocyte cultures where addition of recombinant IL-10 down-regulates the production of TNFα and IL-1, inducing at the same time the release of soluble TNF receptors and IL-1 receptor antagonist [33,34]. Similarly, blocking IL-11 in rheumatoid synovial membrane cultures increases the production of TNFα, whereas addition of recombinant IL-11 inhibits the production of TNFα and matrix metalloproteinases (MMPs) 1 and 3 [35]. Other potentially inhibitory cytokines such as IL-4 are poorly expressed in the rheumatoid synovium, whereas the presence of IL-13 is highly variable.

Soluble forms of the p55- and p75-TNFR are also elevated in RA plasma and synovial fluid, and are spontaneously released in rheumatoid synovial membrane cultures [36], as is the soluble IL-1 receptor and the IL-1ra [37–39]. Although this forms an important group of natural and specific TNFα and IL-1 inhibitors, TNFα and IL-1 bioactivity and signalling (such as NF-κB activation) are still detectable in rheumatoid synovial membrane cultures and in synovial tissue [40,41]. In summary, these observations suggest that anti-inflammatory mechanisms that include the production of IL-10, IL-11, IL-1ra and soluble TNF and IL-1 receptors, are nevertheless insufficient in regulating the disease process. Thus, rheumatoid arthritis may be envisaged as a disease where there is an imbalance between the production of pro-inflammatory and anti-inflammatory mediators (Fig. 1). Various factors such as hormonal changes during

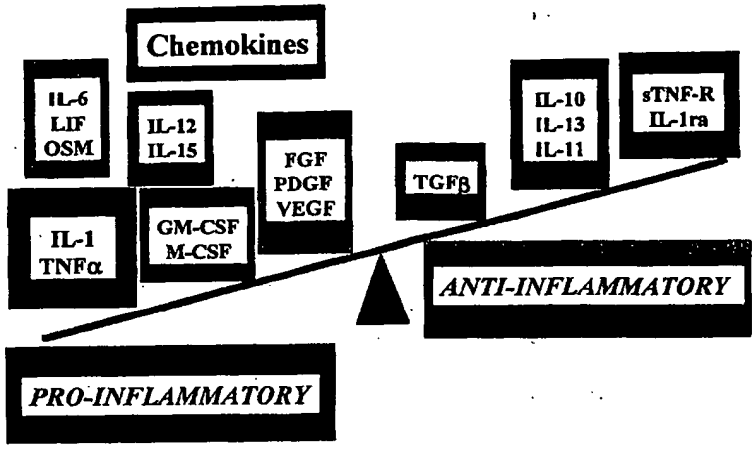


Fig. 1. Cytokine imbalance in the rheumatoid synovium (modified with permission from Feldmann et al. [9]).

late pregnancy and early postpartum may shift this cytokine balance towards one side or another, inducing in that way remissions or exacerbations of disease activity [42].

4. The cytokine system in other autoimmune diseases

Although the cytokine networks of other autoimmune diseases remain less clearly defined than that of rheumatoid arthritis, the expression of cytokines has been studied fairly extensively. First, in Crohn's disease a large number of pro-inflammatory cytokines (TNFα, IFNγ, IL-1, IL-2, IL-6, IL-12) and chemokines (IL-8, MCP-1 and RANTES) were found to be elevated in studies using immunohistochemistry, whole biopsy cultures, or isolated lamina propria mononuclear cells (LPMC) [43–45]. As in rheumatoid arthritis, this was paralleled by an increase in soluble TNF receptors and the IL-1 receptor antagonist [46], and the immunoregulatory cytokine IL-10 [47], but this was insufficient to control the inflammatory process. Administration of exogenous IL-10 could further suppress the elevated pro-inflammatory cytokine production both in vitro in LPMC cultures and in vivo in patients with active disease [48], demonstrating that in Crohn's disease there may also be an imbalance between the production of pro-inflammatory and anti-inflammatory mediators. Although a pro-inflammatory cascade with TNFα at its apex has not been demonstrated in vitro, it is likely to operate in vivo, as anti-TNFα clinical trials have shown marked benefit [49,50].

In Grave's disease and Hashimoto's thyroiditis, pro-inflammatory (TNFα, LT, IL-1, IL-6), immunoregulatory (IL-10) and T cell-derived (IL-2, IL-4, IFNγ) cytokines, as well as chemokines (IL-8) have been detected [51,52]. Most of them are due to the intrathyroid lymphocytic infiltrate seen in thyroid diseases, although thyroid epithelial cells can also produce cytokines [51,53]. Third, in Sjogren's syndrome elevated levels of TNFα, LT, IL-1, IL-2, IL-4, IL-6, IL-10, TGFβ and IFNγ have been detected in salivary gland biopsies by using polymerase chain reaction and in situ hybridisation [54], whereas in systemic lupus erythematosus increased levels of TNFα, IL-1, IL-2, IL-6, IL-10, IFNγ and soluble TNF receptors have been found in sera [55,56]. Finally, in lesions of multiple sclerosis patients with active disease higher levels of TNFα, LT, IL-1, IL-2 and IFNγ have been reported [57]. More detailed reviews about the role of cytokines in various autoimmune diseases can be found in two recent books, '*Cytokines in Autoimmunity*' edited by Brennan and Feldmann [58] and '*Cytokine Reference*' (also on the web at www.apnet.com/cytokinereference) edited by Oppenheim Feldmann [1].

5. Anti-cytokine therapy in rheumatoid arthritis

The laboratory observation that TNFα is at the apex of the pro-inflammatory cascade of rheumatoid arthritis synovial cultures combined with the studies in animal models supporting a role of TNFα for the development and progression of arthritis established TNFα as a target for therapeutic intervention. Clinical trials aimed at blocking TNFα were initiated in 1992 and involved the use of infliximab (Remicade®, initially known as cA2), a chimeric mouse Fv-human IgG1 monoclonal antibody of high TNFα-neutralising capacity produced by Centocor Inc. [59]. The first clinical trial was a Phase I/II open (non-placebo controlled) trial of infliximab in long-standing active RA patients who failed all prior therapy averaging four disease-modifying drugs. The results were very encouraging with rapid alleviation of pain, morning stiffness and tiredness, and reduction of swollen and tender joints within a week or two (Fig. 2a). The serum concentration of inflammatory markers such as C-reactive protein (CRP) were also reduced (Fig. 2b). Although this response was temporary, and lasted 8–22 weeks [60], readministration of infliximab induced further benefit [60,61].

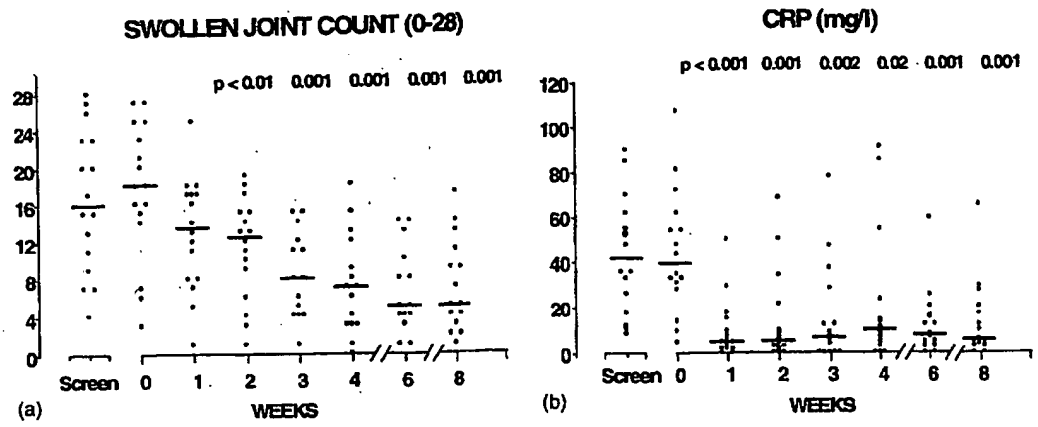


Fig. 2. Open-label treatment of rheumatoid arthritis with infliximab (modified with permission from Elliott et al. [60]). Patients received 20 mg/kg of infliximab (2–4 infusions) within the first 2 weeks of the trial and were subsequently monitored for swollen joints and CRP levels for 8 weeks (taken with permission from Elliott et al. [60]).

The efficacy of infliximab was subsequently confirmed in a Phase II double-blind, randomised, placebo-controlled clinical trial where a 60–70% reduction in the measures of disease activity (such as swollen or tender joint counts and CRP) was observed. In this study, 79% of patients receiving 10 mg/kg and 44% of patients receiving 1 mg/ml of infliximab reached the required level of improvement when compared to 8% of the placebo control patients. This was the formal proof of efficacy. However, the duration of this trial was very short (4 weeks) in order to be able to have patients in placebo not dropping out. Hence, further trials were needed to establish whether longer-term treatment was possible.

Thus, a multidose Phase II clinical trial was done in patients with active disease despite therapy with methotrexate (MTX), one of the most effective and durable treatments. Results from this study demonstrated a synergy between low doses of infliximab and low dose MTX. The onset of the response was rapid with a significant improvement in symptoms and signs by 2 weeks and with most of the patients responding by 6 weeks (Fig. 3).

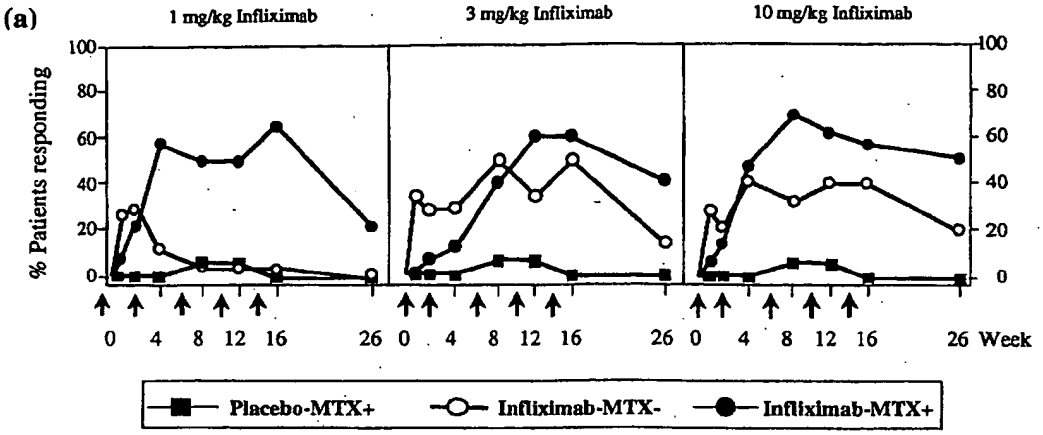
In the similarly designed Phase III trial (ATTRACT), over 60–70% improvement in individual parameters of disease activity was again achieved [62]. This trial was the first of sufficient duration to permit evaluation of the effect of anti-TNFα therapy in joint architecture. Joint protection was observed when hands and feet were examined using a modified sharp X-ray scoring system after 26, 54 and 108 weeks [62,63].

These observations of joint protection with TNFα blockade were reproduced by using a number of other TNFα blocking agents such as etarnecept [64], a p75-TNFR fusion protein and D2E7/adalimumab [65], a human monoclonal antibody, indicating that TNFα blockade is effective at blocking structural damage. This resolves a theoretical discussion based on animal models which predicted that IL-1 blockade would be necessary [66]. The release of the data of the first clinical trial of cA2 in 1992 prompted other companies which had developed TNFα inhibitory biological agents (antibodies or modified TNF receptors) to initiate trials. These agents are summarised in Table 2.

The one that has been most efficient in clinical trials, and in subsequent sales is etanercept (Enbrel®) produced by Immunex/American Home Products. In placebo-controlled Phases II and III trials, etanercept, reduced swollen and tender joins and ameliorated disease according to the ACR criteria [67–69]. Etanercept was also beneficial to patients with active RA despite MTX therapy [68]. Finally, etanercept retarded the progression of bone erosions, as assessed by radiography of hands and feet of patients with active disease of <3 years [64]. D2E7 is also an effective TNFα blocking agent in RA over a range of doses. Subcutaneous or intravenous injections ameliorate disease in a comparable manner to infliximab or etanercept and induce joint protection [65]. For the PEGylated p55-TNFR, only preliminary results from the Phase I/II clinical trials have been

Table 2
TNFα blocking agents used in the clinic in rheumatoid arthritis (modified with permission from Feldmann and Maini [13])

Number	Clinical benefit induced by anti-TNFα treatment
1	Down-regulation of many pro-inflammatory cytokines in vivo IL-1, GM-CSF, IL-6, IL-8 and other chemokines
2	Reduction in leukocyte trafficking Reduction in adhesion molecules and chemokines
3	Reduction in angiogenesis Reduction in VEGF
4	Reduction in joint destruction Reduction in IL-1, MMPs → XR changes
5	Haematological normalisation Haemoglobin ↑, Platelets ↓, Fibrinogen ↓:cardiovascular risk ↓?



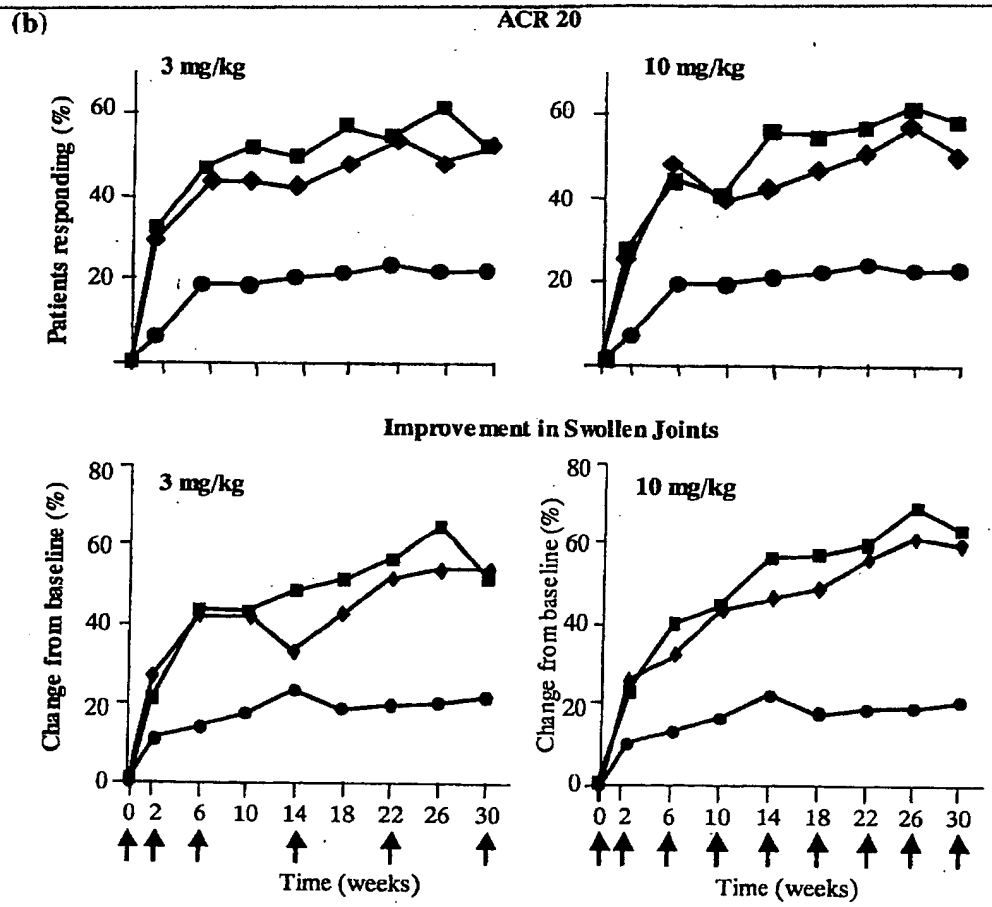


Fig. 3. Efficacy of combination of infliximab and methotrexate vs. methotrexate and placebo. (a) Patients were treated with MTX (7.5 mg per week) and either placebo, 3 or 10 mg/kg infliximab administered intravenously at time points indicated by the arrows. Patients were then followed for disease improvement (modified with permission from Maini et al. [122]). (b) Patients were treated with MTX (15 mg per week) and either placebo, 3 or 10 mg/kg infliximab every 4 or 8 weeks. After 30 weeks, the response of the patients was evaluated with permission from Maini et al. [62]. In both cases, the ACR 20 criteria were used for disease assessment.

Table 3
Clinical benefit induced by anti-TNFα treatment (modified with permission from Feldmann and Maini [13])

Name	Composition	Manufacturer
Monoclonal antibodies		
Infliximab, Remicade®	Chimeric (mouse × human)	Centocor Inc., USA
CDP571	Humanised murine CDR3	Celltech, UK
D2E7, Adalimumab	Fully human	Cambridge Antibody Technology/BASF, UK
PEG Fab	Fully human	Celltech, UK
TNFR:Fc fusion proteins		
Etanercept, Enbrel®	p75-TNFR:Fc	Immunex/American Home Products
Lenercept™	p55-TNFR:Fc	Roche, Switzerland
	PEGp55-TNFR	Amgen, USA

published and look encouraging [70], whereas for lenercept, a p55-TNFR fusion protein, results have been positive but somewhat variable probably due to immunogenicity of the construct or manufacturing problems and batch to batch variation [71]. In summary, though, the consistent beneficial effect of multiple TNFα blocking agents formally confirmed the essential role of TNFα in RA. It is, thus, important to understand what drives the deregulated TNFα production in the rheumatoid synovium.

Anti-cytokine therapy in RA is not limited to TNFα blockade. Other important pro-inflammatory cytokines have also been targeted. One such cytokine is IL-1. When IL-1 was blocked by administering IL-1ra (Anakinra®, Amgen) in a multicenter, double-blind, randomised, controlled study, results were positive but less dramatic than these of the anti-TNFα trials. They revealed an improvement in clinical disease as judged by the ACR criteria [72] and in joint destruction as determined by radiology [72–74]. This may be indicative of an important role of IL-1 in bone resorption, already suggested by both in vitro and in vivo studies [15,75]. This agent has recently been approved by the Food and Drug Administration (FDA) in the USA for the treatment of RA. In animal models, there is evidence that blockade of TNFα and IL-1 is synergistic [76], so it is possible that this may also be the case in humans. Such clinical trials are in progress.

IL-6 has also been targeted in RA. In one study, administration of a murine anti-human IL-6 neutralising antibody demonstrated a short-term improvement in clinical disease although the number of patients used was small [77]. In another open-label study, administration of a humanised monoclonal anti-IL-6 receptor antibody improved the clinical symptoms of RA and normalised acute phase proteins within 2 weeks [78]. Although the overall benefit of anti-IL-6 therapy was slower in onset than that seen with TNFα blockade, it was significant, and randomised trials are underway to provide more information on its effects on inflammation and also in joint erosions and bone loss.The mechanism of action of anti-TNFα blockade has been studied in clinical trials using serum and synovial samples (Table 3). The first question that was addressed was the effect of anti-TNFα in the expression of other cytokines. Anti-TNFα rapidly decreased the levels of cytokines such as IL-1, IL-6, IL-8, MCP-1 and VEGF in the serum [60,79–82]. Smaller studies also demonstrated reductions in synovial cytokines by immunohistology. These results verify that TNFα is indeed at the apex of the inflammatory cascade in vivo, confirming our previous observations in vitro [14,23]. Second, anti-TNFα reduced the trafficking of leukocytes into the joints, directly demonstrated by using labelled cells [81]. This was due to down-regulation of the production of multiple chemokines and adhesion molecules [80,81,83]. Third, anti-TNFα inhibited angiogenesis in inflamed joints [84], a finding that may be partly due to the decrease in VEGF production [80]. Finally, anti-TNFα reduced haematological abnormalities observed in RA patients such as anemia and elevated platelet counts through an unknown mechanism [85]. It is not known at the moment whether blockade of other cytokines can also affect these aspects of the disease, but it is likely that the widespread clinical effects are due to TNFα blockade interfering with multiple biological pathways.

6. Anti-cytokine therapy in other autoimmune diseases

The success of anti-TNFα in RA reported in October 1992 catalysed the organisation and execution of clinical trials in a number of other autoimmune diseases in which similar mechanisms may operate. The first was in Crohn's disease, a chronic inflammatory disease where TNFα is also elevated together with many other cytokines. In an initial Phase I/II open-label study, a single infusion of the anti-TNFα blocking agent infliximab induced clinical and endoscopic improvement in 80% of the patients with active steroid-resistant disease [49] that persisted in 41% of the infliximab-treated patients compared to 12% of placebo control patients [86]. In this study, infliximab reduced the endoscopic disease activity and the inflammatory response in the mucosal layer [87–89]. In the light of the trials in RA, the effect of multiple infusions of infliximab were also examined. It was found that in patients that initially responded to a single infusion of infliximab, 51% of the patients remained in remission for the duration of the study, compared to 21%

placebo controls [50,89]. Infliximab obtained FDA approval for its use in humans for the treatment of Crohn's disease in 1998, due to the fact that it resulted in over 58% fistulas healing in the majority of Crohn's fistula patients [90].

An interesting observation made during treatment of Crohn's disease patients with infliximab was that spondyloarthropathies associated with the disease were also improved [91]. This was paralleled by two open-label pilot studies in spondyloarthropathy patients (e.g. patients with ankylosing spondylitis and psoriatic arthritis), where infliximab was shown to be effective in resistant disease, to improve both axial and peripheral arthritis, and to require multiple infusions for maintenance of the benefit [92–94]. Recently, these observations have also been confirmed in a similarly designed double-blind placebo-controlled study [94]. Notably, infliximab treatment reduced the thickness of the synovial lining layer, the vascularity, the activation of the endothelium and the extravasation of neutrophils and macrophages [95]. As this is reminiscent of the effects of infliximab in RA, it suggests that TNFα is also of critical importance in these diseases. Other TNFα blocking agents such as etanercept are effective in spondyloarthropathies as well [96].

The efficacy of etanercept has also been tested in juvenile arthritis and psoriasis. In a Phase I/II clinical trial in juvenile arthritis, etanercept improved clinical disease in 74% of the patients compared to placebo [97]. This led to its FDA approval for this indication. In another placebo-controlled trial in patients with psoriasis, etanercept also diminished disease activity and severity [98]. In both studies, psoriatic arthritis was also reduced in these patients. Currently, clinical trials are in progress to evaluate the effect of TNFα blocking agents in the treatment of various other diseases such as Wegener's granulomatosis [99], adult onset Still's disease [100], polymyositis [101] and systemic sclerosis [102]. Although data are too preliminary for conclusions to be made, they are encouraging and indicate that TNFα may be important in the pathogenesis of these diseases too. At this point, it should be stressed that anti-TNFα treatment is not beneficial to all autoimmune diseases as trials in multiple sclerosis have not been encouraging. The administration of the TNFα blocking agent lenercept had no effect on brain leakage of patients assessed by gadolinium MRI scoring, the primary endpoint, but increased the frequency of relapses, the secondary endpoint of the trial [103]. The administration of infliximab resulted in an increased gadolinium vascular leak in the brain of two patients, although it is impossible to interpret results in such a limited number of patients except to urge caution [104]. It is interesting from the cytokine biology point of view to speculate why MS is an exception. Our belief is that the benefit of anti-TNFα involves major effects on TNFα at the site of the disease. The blood brain barrier does not permit significant leakage of the large anti-TNFα biologicals (M_w > 150 kDa) into the lesions in the brain, and hence, there is no benefit. There is now considerable evidence that chronic exposure to TNFα inhibits T cell function as it interferes with T cell receptor signalling [105,106]. This implies that TNFα reduction in the periphery of MS patients may augment relapses as the T cell response would be augmented. TNFα can down-regulate IL-12 production too, so this may also favour relapses.

7. Anti-inflammatory cytokine therapy in autoimmunity

An alternative approach to the anti-cytokine therapy to inhibit pro-inflammatory cytokine production involves the use of immunoregulatory cytokines such as IL-4, IL-10, IL-11 and IFNβ. In RA, the efficacy of IL-10 or IL-11 has been tested. In two independent Phase I trials, administration of IL-10 or IL-11 to patients with active disease demonstrated no significant clinical improvement when compared to placebo [107–109]. However, in an open-label study in psoriasis, administration of IL-10 did ameliorate clinical disease in nine out of 10 patients [110]. In a double-blind placebo-controlled study that followed, clinical benefit was mainly observed in the skin but not the arthritic disease activity [111,112]. This was due to the anti-inflammatory properties of IL-10 as IL-10-treated patients had reduced T cell and macrophage infiltration and angiogenesis in the synovium, and suppressed monocyte and T_{H1} function [112].

In contrast, the use of IFNβ in MS has been met with success. Three preparations of IFNβ have been documented to provide benefit, Betaseron® (IFNβ-1b), Avonex® (IFNβ-1a) and Rebif® (IFNβ-1a). In a Phase III double-blind placebo-controlled trial of patients with relapsing–remitting MS, Betaseron® reduced MS attack frequency by 34% over the first 2 years and by approximately 30% over 5 years [113,114]. Severe MS attacks and mean attack duration were also reduced, as was steroid usage and MS-related hospitalisations. Annual MRI scans and over a 5 years period showed up to 85% reduction in disease activity (as measured by the plaque number and plaque size) [115,116]. Betaseron® induced benefit in secondary progressive MS too [117]. In a 2 years placebo-controlled trial, Betaseron® reduced attack frequency by 33% and lengthened the median time of progression of disability by 38%. Betaseron® corrects several immune system abnormalities observed in MS such as increased IFNγ production and HLA-DR expression of circulating blood cells, and deficient function of CD8 regulatory T lymphocytes [118], but whether this can explain its efficacy is not clear.

Anovex® and Rebif® were also found to be beneficial to MS patients in placebo-controlled trials [119,120]. They both reduce attack frequency, disease activity and progression of disability in relapsing–remitting disease. In a recently published double-blind, randomised, multicenter placebo-controlled study, Rebif® was also shown to reduce lesion progression and disease burden in progressive multiple sclerosis [121]. However, and although the results from the IFNβ trials reinforce each other, it has been difficult to

make direct comparisons about their efficacy as the design of these trials (such as patient selection, primary endpoints, etc.) differed. IFNβ preparations have now been approved for human use and are now a standard therapy for multiple sclerosis.

8. Future prospects

How can we progress from the current anti-cytokine therapy to develop novel therapies of increased efficacy? Clearly with respect to RA, anti-TNFα works well as a monotherapy but optimal results are obtained when anti-TNFα blocking agents are administered in combination with MTX [62,63,68,122]. There is increased efficacy and less side effects. This suggests that combination therapy may be a good approach to improve anti-TNFα in the future. In experiments that we have performed in animal models of arthritis, the benefit from anti-TNFα therapy is further increased when combined with anti-T cell therapy. For example, co-administration of neutralising anti-TNFα antibodies with depleting anti-CD4 or anti-CD3 antibodies, or CTLA-4Ig fusion proteins, further ameliorates disease when compared with the anti-TNFα treatment only [123] (Williams, unpublished data). Thus, co-administration of T cell-depleting or T cell function-inhibitory agents may also benefit human RA. Several anti-rheumatic agents including cyclosporin and leflunomide that are known to be inhibitory to T cells [124,125] are candidates for combination therapy. Combination therapy has extensively been reviewed elsewhere [126].

The major drawback of anti-TNFα therapy and consequently additional therapies based on TNFα blocking agents, is the cost of treatment. The inconvenience of administering anti-TNFα by injection, and the risk of increasing infections after chronic treatment are also drawbacks [127]. To design better therapeutic agents of lower cost and more convenient administration routes that specifically block the production of pathological TNFα without compromising the expression of physiological TNFα and immunoregulatory cytokines, we decided to investigate the intracellular signalling pathways that regulate TNFα but also other cytokines. We envisaged that at some stage, the mechanisms leading to TNFα production would differ between pathological and normal physiological conditions.

First, we examined the role of NF-κB, an important transcription factor that can bind to the 5′ promoter region of the TNFα gene as can AP-1, NF-IL-6 and NF-AT. Using recombinant adenoviruses as an efficient gene transfer technique to rheumatoid synovial cells that include macrophages, T cells

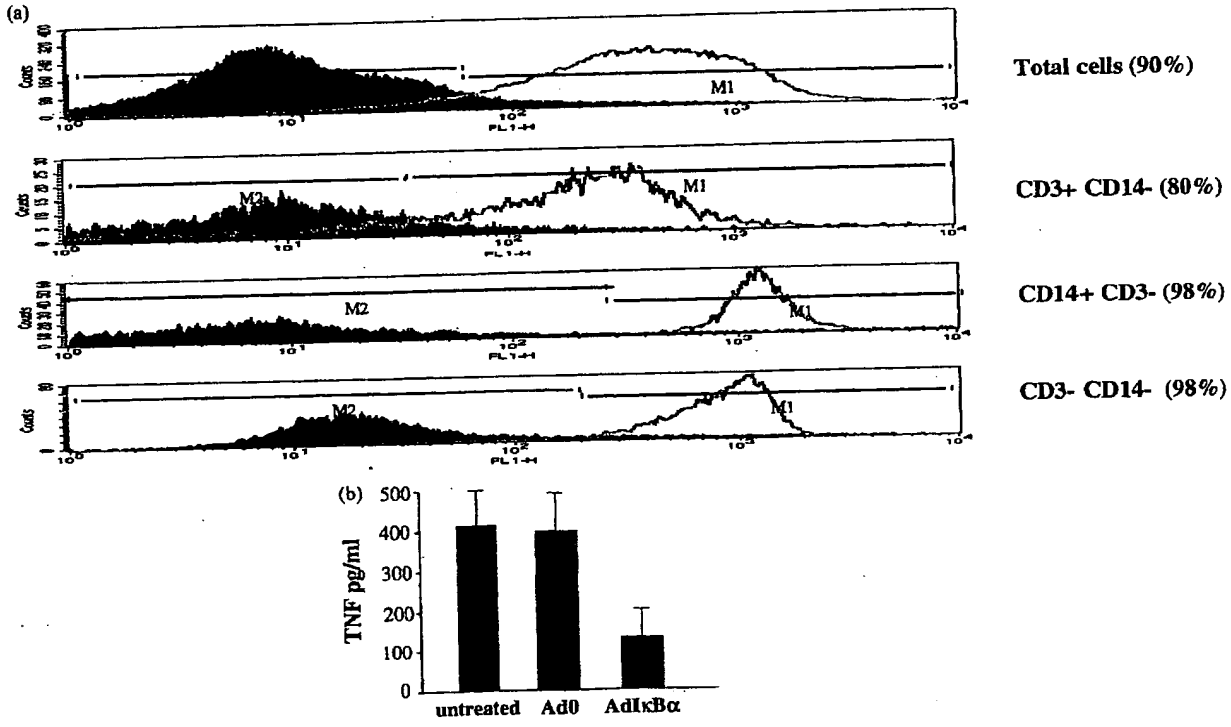


Fig. 4. TNFα production in rheumatoid synovial cell cultures is NF-κB-dependent. (a) More than 90% of rheumatoid synovial cells can be infected with replication-deficient adenoviruses as shown by measuring β-galactosidase activity by FACS. Rheumatoid T cells, macrophage- and fibroblast-like cells are all efficiently infected (modified with permission from Bondeson et al. [129,130]). (b) Infection of rheumatoid synovial cells with an adenovirus overexpressing IκBα (AdIκBα) but not a control adenovirus without insert (Ad0) inhibits TNFα production (modified with permission from Foxwell et al. [128]).

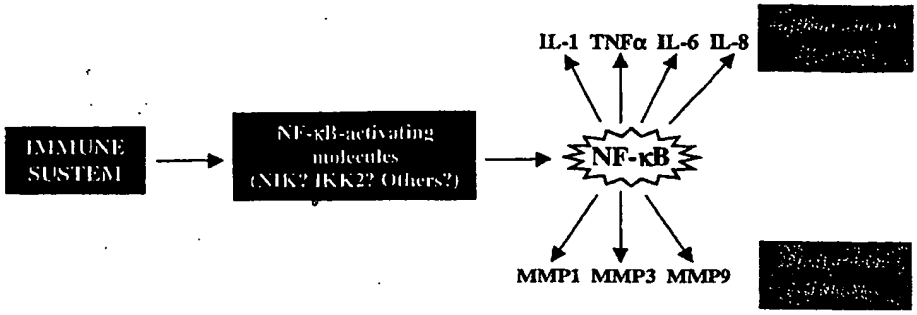


Fig. 5. NF-κB is central to the regulation of inflammatory and destructive processes in the rheumatoid synovium. Therapeutics aimed at blocking NF-κB or upstream NF-κB-activating molecules constitute a promising new approach for the treatment of RA and possibly other chronic inflammatory diseases.

and fibroblasts [128,129], we found that overexpression of IκBα, the natural inhibitor of NF-κB, inhibited TNFα production by 70% [128,129]. This suggested that NF-κB is rate-limiting for TNFα production in the rheumatoid synovium, an observation that is not always the case in other cells [130]. Interestingly, although NF-κB is also involved in the expression of other pro-inflammatory cytokines such as IL-1, IL-6 and IL-8, it is only minimally involved in the production of the immunoregulatory cytokines IL-10 and IL-11, or the release of soluble TNF receptors (Fig. 4).

Similarly, NF-κB is required for the expression of MMP-1, MMP-3 and MMP-13, enzymes that play a role in the destructive process of RA by breaking down human cartilage [131–133], but not TIMP-1, the inducible tissue inhibitor of MMP enzymatic action. Although it is not clear whether the effect of NF-κB inhibition on MMP production is due to a direct role of NF-κB on MMP gene expression or an indirect effect on cytokines inducing MMP expression, these findings demonstrate that NF-κB is essential for both inflammatory and destructive processes of RA. As NF-κB is only minimally involved in the regulation of anti-inflammatory cytokines or TIMP-1, blocking NF-κB may be beneficial in RA as it may restore the cytokine equilibrium in the joint by reducing at the same time cartilage and bone damage (Fig. 5). Similar data have also been obtained from animal models of arthritis [134,135], providing compelling evidence about the role of NF-κB in inflammatory arthritis. Indeed, many conventional anti-inflammatory and anti-arthritic agents such as glucocorticoids, sodium salicylate and sulfosalazine are inhibitors of NF-κB and TNFα, suggesting that this could at least partially explain their therapeutic efficacy [136,137].

The means to inhibit NF-κB with specificity and safety are not yet available, but as many companies are working on NF-κB inhibitors, this may change in the near future. Determining the upstream signalling pathways that lead to NF-κB activation in disease states may also contribute to safety as it may allow the specific targeting of the pathological NF-κB-activating molecules. In that respect, we have recently found that NF-κB-inducing kinase (NIK) is not involved in TNFα production in dissociated synovial membrane cultures [138] or other inflammatory disease tissue such as bronchoalveolar lavage tissue from fibrosing alveolitis patients [139], and we are currently investigating the involvement of other putative NF-κB-activating molecules in that process.

Another major pathway that controls inflammatory gene expression and is of relevance in therapy involves the action of mitogen-activated protein kinases (MAPKs). In human monocytes/macrophages, p54 MAPK controls TNFα production at the translational level [140], whereas p42/44 MAPK regulates TNFα production at the transcriptional levels [141,142]. On the other hand, p38 MAPK affects TNFα expression at multiple levels that involve transcriptional, post-transcriptional and translational mechanisms [142–144]. In rat models of arthritis, PRP200765A, a novel p38 MAPK inhibitor reduces the incidence and progression of arthritis [145], whereas SP600125, a novel p54 MAPK inhibitor prevents radiological joint destruction but only modestly decreases paw swelling [146]. For optimal therapeutic efficacy, combinations of inhibitors of different MAPKs may be required. Clinical trials of p38 MAPK inhibitors in RA are underway, so their efficacy in humans will be evaluated soon.

9. Conclusions

The identification, cloning and study of cytokines and cytokine networks over the last two decades provided a novel family of therapeutic targets for the treatment of a whole range of autoimmune diseases. This is now being translated to therapeutic benefit with TNFα blocking biologicals being approved and extensively used to treat rheumatoid arthritis and Crohn's disease patients (with >200,000 patients treated by the end of 2001), and looking promising for the treatment of other related diseases such as psoriasis and spondyloarthropathies. Durability of the benefit, safety and pharmacoeconomic issues will determine whether this early success will prove to be a major breakthrough to treatment of these painful and incurable diseases. The impetus to further research of these successes augurs well for the future.

Acknowledgements

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INFLIXIMAB FOR THE TREATMENT OF FISTULAS IN PATIENTS WITH CROHN'S DISEASE

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ABSTRACT

Background Enterocutaneous fistulas are a serious complication of Crohn's disease and are difficult to treat. Infliximab, a chimeric monoclonal antibody to tumor necrosis factor α, has recently been developed as a treatment for Crohn's disease. We conducted a randomized, multicenter, double-blind, placebo-controlled trial of infliximab for the treatment of fistulas in patients with Crohn's disease.

Methods The study included 94 adult patients who had draining abdominal or perianal fistulas of at least three months' duration as a complication of Crohn's disease. Patients were randomly assigned to receive one of three treatments: placebo (31 patients), 5 mg of infliximab per kilogram of body weight (31 patients), or 10 mg of infliximab per kilogram (32 patients); all three were to be administered intravenously at weeks 0, 2, and 6. The primary end point was a reduction of 50 percent or more from base line in the number of draining fistulas observed at two or more consecutive study visits. A secondary end point was the closure of all fistulas.

Results Sixty-eight percent of the patients who received 5 mg of infliximab per kilogram and 56 percent of those who received 10 mg per kilogram achieved the primary end point, as compared with 26 percent of the patients in the placebo group ($P=0.002$ and $P=0.02$, respectively). In addition, 55 percent of the patients assigned to receive 5 mg of infliximab per kilogram and 38 percent of those assigned to 10 mg per kilogram had closure of all fistulas, as compared with 13 percent of the patients assigned to placebo ($P=0.001$ and $P=0.04$, respectively). The median length of time during which the fistulas remained closed was three months. More than 60 percent of patients in all the groups had adverse events. For patients treated with infliximab, the most common were headache, abscess, upper respiratory tract infection, and fatigue.

Conclusions Infliximab is an efficacious treatment for fistulas in patients with Crohn's disease. (N Engl J Med 1999;340:1398-405.)

CROHN'S disease is a chronic inflammatory bowel disease of unknown cause, which is characterized by segmental transmural inflammation and granulomatous lesions of the intestinal mucosa. The disease is complicated by the development of fistulas in approximately one third of patients.[1] Fistulas may be internal (e.g., bowel to bowel, bowel to bladder, or rectovaginal) or enterocutaneous (extending through the abdominal wall or into the perineum). These fistulas rarely heal spontaneously or as a result of drug treatment and frequently require surgery. According to anecdotal evidence, antibiotics have short-term efficacy in their treatment. Although the use of immunomodulatory agents is associated with improvement and closure of fistulas, no significant effect has been demonstrated in prospective, placebo-controlled studies.[2-4]

The local production of tumor necrosis factor α (TNF-α) is thought to have a key role in the initiation and propagation of Crohn's disease.[5-7] Production of TNF-α in the intestinal mucosa is increased in patients with Crohn's disease.[7-9] Neutralization of TNF-α has been suggested as a therapeutic intervention in inflammatory diseases, such as inflammatory bowel disease and rheumatoid arthritis.[5,10]

Infliximab (formerly known as cA2) is a genetically constructed IgG1 murine–human chimeric monoclonal antibody that binds both the soluble subunit and the membrane-bound precursor of TNF-α.[11,12] Infliximab inhibits a broad range of biologic activities of TNF-α, presumably by blocking the interaction of TNF-α with its receptors, and it may also cause lysis of cells that produce TNF-α.[12,13] Infliximab has been found to be efficacious and safe in the treatment of moderate-to-severe Crohn's disease in several clinical trials.[14-17] Anecdotal reports of the closure of fistulas in these trials prompted us to evaluate the efficacy of infliximab in healing enterocutaneous fistulas.

METHODS

Patients

We enrolled patients who were 18 to 65 years of age and who had single or multiple draining abdominal or perianal fistulas of at least three months' duration as a complication of Crohn's disease that had been confirmed by radiography, endoscopy, or pathological examination. Patients could receive concomitant therapy. Acceptable regimens were aminosalicylates at a dosage that had been stable for more than four weeks before screening; oral cortico-

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Other participants in the study are listed in the Appendix.

steroids at a dosage of 40 mg or less per day that had been stable for more than three weeks; methotrexate given for at least three months at a dosage that had been stable for more than four weeks; azathioprine or mercaptopurine given for at least six months at a dosage that had been stable for more than eight weeks; and antibiotics at a dosage that had been stable for more than four weeks. If patients were not currently receiving treatment with any of these medications, they had to have discontinued therapy at least four weeks before enrollment. Patients treated concurrently with cyclosporine were excluded from the study. Treatment with investigational agents or the use of any medication to reduce the concentration of TNF-α was not allowed within three months before enrollment. Additional exclusion criteria were other complications of Crohn's disease, such as current strictures or abscesses; the presence of a stoma created less than six months before enrollment; a history of allergy to murine proteins; and previous treatment with infliximab. Men and women with reproductive potential were required to use an acceptable form of birth control throughout the study and for six months after the final infusion.

One hundred twenty patients were screened at 12 centers in the United States and Europe, of whom 94 were enrolled. The protocol was approved by the institutional review boards and ethics committees at all sites, and all patients gave written informed consent before enrolling in the study.

Protocol

The screening procedures included a physical examination, routine laboratory analyses, assessment of the severity of disease according to the Crohn's Disease Activity Index and, for patients who had perianal disease at base line, a Perianal Disease Activity Index. The Crohn's Disease Activity Index incorporates eight related variables: the number of liquid or very soft stools per day, the severity of abdominal pain or cramping, general well-being, the presence or absence of extraintestinal manifestations of disease, the presence or absence of an abdominal mass, the use or nonuse of antidiarrheal drugs, the hematocrit, and body weight.[18] These items yield a composite score ranging approximately from 0 to 600; scores below 150 indicate remission, whereas scores above 450 indicate severe illness. The Perianal Disease Activity Index incorporates five elements: the presence or absence of discharge, pain or restriction of activities of daily living, restriction of sexual activity, the type of perianal disease, and the degree of induration, yielding a composite score ranging from 0 to 20, with higher scores indicating more severe disease.[19] All fistulas had to be distinctly identifiable; drawings as well as photographs were used to document the sites of disease.

Within seven days of screening, eligible patients were randomly assigned to receive one of three treatments: placebo, 5 mg of infliximab per kilogram of body weight, or 10 mg of infliximab per kilogram, all to be given intravenously at weeks 0, 2, and 6. Randomization was performed by an independent organization (PPD Pharmaco, Austin, Tex.), using a stratified treatment assignment[20] with the investigational site and the number of fistulas (one or more than one) as the stratification variables. Patients were enrolled from May 30 through October 1, 1996.

Infliximab was administered intravenously. Infliximab (Chimeric A2 [cA2] IgG, Centocor, Malvern, Pa.) was supplied as a lyophilized solid containing 250 mg of cA2 IgG, 2.5 g of sucrose, 61.0 mg of dibasic sodium phosphate dihydrate, 21.7 mg of monobasic sodium phosphate monohydrate, and 2.5 mg of polysorbate 80 in a 100-ml vial for reconstitution in 50 ml of sterile water. The medication was added to the diluent directly from the 100-ml vial with a 15-μm filter, then infused slowly over a two-hour period.

The placebo preparation was supplied as a lyophilized solid containing 25 mg of human serum albumin, 2.5 g of sucrose, 61.0 mg of dibasic sodium phosphate dihydrate, 21.7 mg of monobasic sodium phosphate monohydrate, and 2.5 mg of polysorbate 80 in a 100-ml vial for reconstitution in 50 ml of sterile water. The placebo was identical in appearance to the infliximab solution.

After the first infusion of study medication, patients returned for clinical and laboratory assessments at weeks 2, 6, 10, 14, and 18. Blood samples were drawn at each study visit and at weeks 26 and 34 to determine the serum concentration of infliximab.

Evaluation of Efficacy

The primary efficacy end point was defined a priori as a reduction of 50 percent or more from base line in the number of draining fistulas observed at two or more consecutive study visits. Treatment was considered to have failed in patients who had changes in medication that were not permitted in the protocol, who underwent surgery related to Crohn's disease, or who did not return for follow-up visits.

The primary end point was based on the investigators' physical evaluation of the patient; a fistula was considered to be closed when it no longer drained despite gentle finger compression. Draining fistulas of less than three months' duration at base line were excluded from the primary analysis. In order for a patient to reach the primary end point, a minimum of 21 days between consecutive visits was required.

Secondary analyses of efficacy evaluated the number of patients with a complete response (defined as the absence of any draining fistulas at two consecutive visits), the length of time to the beginning of a response, and the duration of the response. Changes in scores on the Crohn's Disease Activity Index and the Perianal Disease Activity Index were also evaluated.

Evaluation of Safety

Safety was assessed in terms of the incidence of adverse events and changes in vital signs and routine laboratory measures. Patients were monitored for adverse events during each infusion and at each study visit.

Immunologic Evaluation

We conducted assays to detect the formation of antinuclear antibodies, antibodies against double-stranded DNA, and human antichimeric antibodies. Antinuclear antibodies were measured by means of a standard immunofluorescence technique in HEp-2 cells, with a screening dilution of the sample of 1:40 (negative results were defined as less than 1:40). Patients who were positive for antinuclear antibodies were evaluated for antibodies against double-stranded DNA with the *Crithidia luciliae* immunofluorescence technique and a screening dilution of 1:10. Antibodies against double-stranded DNA were measured in patients with positive results by means of the Farr radioimmunoassay. Patients were considered positive for antibodies against double-stranded DNA if they had positive results on both the *C. luciliae* immunofluorescence assay and the Farr radioimmunoassay. Human antichimeric antibodies were measured with use of a double-antigen enzyme immunoassay.

Statistical Analysis

The primary analysis was performed according to the intention-to-treat principle and included all patients who were screened and randomly assigned to treatment. The analysis was performed in two stages. We performed the Mantel–Haenszel chi-square test for a linear dose response in the proportion of patients in whom the primary end point occurred. If the result was significant at an alpha level of 0.05, Fisher's exact test was then used to compare the proportion of patients achieving the primary end point in each of the two infliximab groups with that in the placebo group. Odds ratios were used to assess the consistency of benefit of infliximab treatment in subgroups of patients.

Analysis of the proportion of patients who had a complete response was performed with the same methods used for the analysis of the primary end point. Continuous variables (e.g., scores on the Crohn's Disease Activity Index and Perianal Disease Activity Index) were compared by analysis of variance of the van der Waerden normal scores. For patients who discontinued regularly scheduled follow-up, underwent a surgical procedure, or had a change in medication that was not permitted by protocol, the measurements

from the last evaluation were carried forward. All reported P values are two-sided.

RESULTS

Ninety-four patients were randomly assigned to treatment with infliximab or placebo. Demographic and clinical characteristics and rates of use of concomitant medications were similar in all treatment groups at base line (Table 1). Six patients discontinued treatment (four in the placebo group and two treated with infliximab); all had received two of the three scheduled infusions. The reasons for withdrawal were lack of efficacy (three patients in the placebo group), administrative reasons (one in the placebo group), withdrawal of consent (one patient assigned to 5 mg of infliximab per kilogram), and adverse events (one patient treated with 10 mg of infliximab per kilogram).

Efficacy

With respect to the primary efficacy end point, response rates were significantly greater among the patients receiving infliximab (68 percent in the group assigned to 5 mg per kilogram and 56 percent in the group receiving 10 mg per kilogram) than in the placebo group (26 percent; $P=0.002$ and $P=0.02$, respectively) (Table 2). Response rates in the two infliximab groups were not significantly different ($P=0.35$). The results of treatment are summarized in Table 2. Photographs of the healing of fistulas over time in two patients are shown in Figure 1. There was a complete response, defined as the absence of any draining fistulas, in 55 percent of the patients treated with 5 mg of infliximab per kilogram, in 38 percent of those treated with 10 mg per kilogram, and in 13 percent of patients receiving placebo ($P=0.001$ and $P=0.04$, respectively). Complete responses occurred both in patients with single fistulas and in those with multiple fistulas; of the 29 infliximab-treated patients with a complete response, 15 had a single fistula and 14 had multiple fistulas at base line.

In patients who reached the primary end point, the length of time to the beginning of a response was calculated as the number of days from the initial infusion to the first of the two or more consecutive

TABLE 1. BASE-LINE CHARACTERISTICS OF THE PATIENTS, ACCORDING TO STUDY GROUP.*

CHARACTERISTIC	PLACEBO (N=31)	INFLIXIMAB		ALL PATIENTS (N=94)
		5 mg/kg (N=31)	10 mg/kg (N=32)	
Age — yr	35.4±8.6	41.2±12.2	35.0±12.3	37.2±11.4
Weight — kg	69.4±12.0	70.4±14.5	66.2±15.0	68.6±13.9
Race — no. (%)				
White	29 (94)	28 (90)	29 (91)	86 (91)
Black	2 (6)	3 (10)	3 (9)	8 (9)
Sex — no. (%)				
Male	17 (55)	15 (48)	12 (38)	44 (47)
Female	14 (45)	16 (52)	20 (62)	50 (53)
Duration of Crohn's disease — yr	12.0±7.9	13.6±9.5	11.5±8.2	12.4±8.5
Area of involvement — no. (%)				
Ileum	3 (10)	7 (23)	4 (12)	14 (15)
Colon	9 (29)	7 (23)	10 (31)	26 (28)
Ileum and colon	19 (61)	17 (55)	18 (56)	54 (57)
Previous segmental resection — no. (%)	12 (39)	21 (68)	17 (53)	50 (53)
No. of enterocutaneous fistulas — no. (%)				
1	13 (42)	15 (48)	14 (44)	42 (45)
>1	18 (58)	16 (52)	18 (56)	52 (55)
Location of fistula				
Perianal	29 (94)	27 (87)	29 (91)	85 (90)
Abdominal	2 (6)	4 (13)	3 (9)	9 (10)
Previous medication — no. (%)				
Corticosteroids	11 (35)	12 (39)	10 (31)	33 (35)
Mercaptopurine or azathioprine	9 (29)	12 (39)	17 (53)	38 (40)
Aminosalicylates	19 (61)	17 (55)	16 (50)	52 (55)
Antibiotics	11 (35)	6 (19)	11 (34)	28 (30)
Score on Crohn's Disease Activity Index†	192.9±92.0	184.4±98.5	184.9±97.5	187.3±95.0

*Plus–minus values are means ±SD.

†Data were available for 25 patients in the placebo group, 27 assigned to 5 mg of infliximab per kilogram, and 27 assigned to 10 mg per kilogram (total, 79 patients).

TABLE 2. OUTCOME OF TREATMENT, ACCORDING TO STUDY GROUP.

VARIABLE	PLACEBO	INFLIXIMAB		
		5 mg/kg	10 mg/kg	5 OR 10 mg/kg
End points				
Primary end point — no./total no. (%)*	8/31 (26)	21/31 (68)	18/32 (56)	39/63 (62)
P value vs. placebo	—	0.002	0.02	0.002
Complete response — no./total no. (%)†	4/31 (13)	17/31 (55)	12/32 (38)	29/63 (46)
P value vs. placebo	—	0.001	0.04	0.001
Time to onset of response (days)‡				
Median	42	14	14	14
Interquartile range	15–72	14–42	14–42	14–42
Duration of response (days)‡				
Median	86	84	99	86
Interquartile range	56–104	31–113	86–113	57–113
Crohn's Disease Activity Index§				
Base-line score				
Median	162	163	203	168
Interquartile range	126–265	99–284	112–254	112–258
P value vs. placebo	—	0.71	0.66	0.64
Score at week 2				
Median	171	108	111	108
Interquartile range	114–252	83–203	89–164	84–179
P value vs. placebo	—	0.04	0.06	0.02
Score at week 18				
Median	160	104	123	117
Interquartile range	72–206	47–177	58–175	49–177
P value vs. placebo	—	0.23	0.32	0.21
Perianal Disease Activity Index¶				
Base-line score				
Median	9.0	8.0	10.0	9.0
Interquartile range	7.0–10.5	7.0–10.0	8.0–12.0	7.0–11.0
P value vs. placebo	—	0.69	0.31	0.73
Score at week 2				
Median	8.0	6.0	6.0	6.0
Interquartile range	6.0–10.0	3.0–7.0	4.0–8.0	3.5–8.0
P value vs. placebo	—	0.02	0.04	0.01
Score at week 18				
Median	7.0	4.0	5.0	5.0
Interquartile range	4.0–9.0	1.0–7.0	3.0–8.0	2.0–7.5
P value vs. placebo	—	0.05	0.14	0.05

*The primary end point was defined as a reduction of 50 percent or more from base line in the number of open fistulas observed at two or more consecutive visits.

†A complete response was defined as the absence of any draining fistulas at two or more consecutive visits.

‡Only patients achieving the primary end point were included.

§The Crohn's Disease Activity Index is based on eight variables (described in the Methods section). Scores range approximately from 0 to 600; scores below 150 indicate remission, and scores above 450 indicate severe illness. Values shown are based on 79 of the 94 patients; patients with a stoma were excluded because the number of stools could not be assessed.

¶The Perianal Disease Activity Index is based on five variables. Scores range from 0 to 20, with higher scores indicating more severe disease.

visits at which this end point was observed. The median time to the onset of a response (Table 2) was shorter among patients treated with infliximab (two weeks) than among those given placebo (six weeks). The duration of the response was defined as the maximal period during which the patient had a reduction of 50 percent or more in the number of draining fistulas at consecutive visits. The median duration of response was approximately three months in patients who reached the primary end point (Table 2). Changes over time in the scores on the Crohn's Disease Activity Index and the Perianal Disease Activity Index are shown in Table 2, according to treatment group.

A consistent benefit of infliximab treatment was observed for all demographic subgroups we evaluated (Table 3). A significant benefit of treatment was still evident when the analyses were adjusted for sex or prior bowel resection with logistic regression ($P=0.001$, data not shown). Significantly more of the patients with single fistulas who were treated with infliximab reached the primary end point than patients assigned to placebo (52 percent vs. 8 percent, $P=0.02$); the

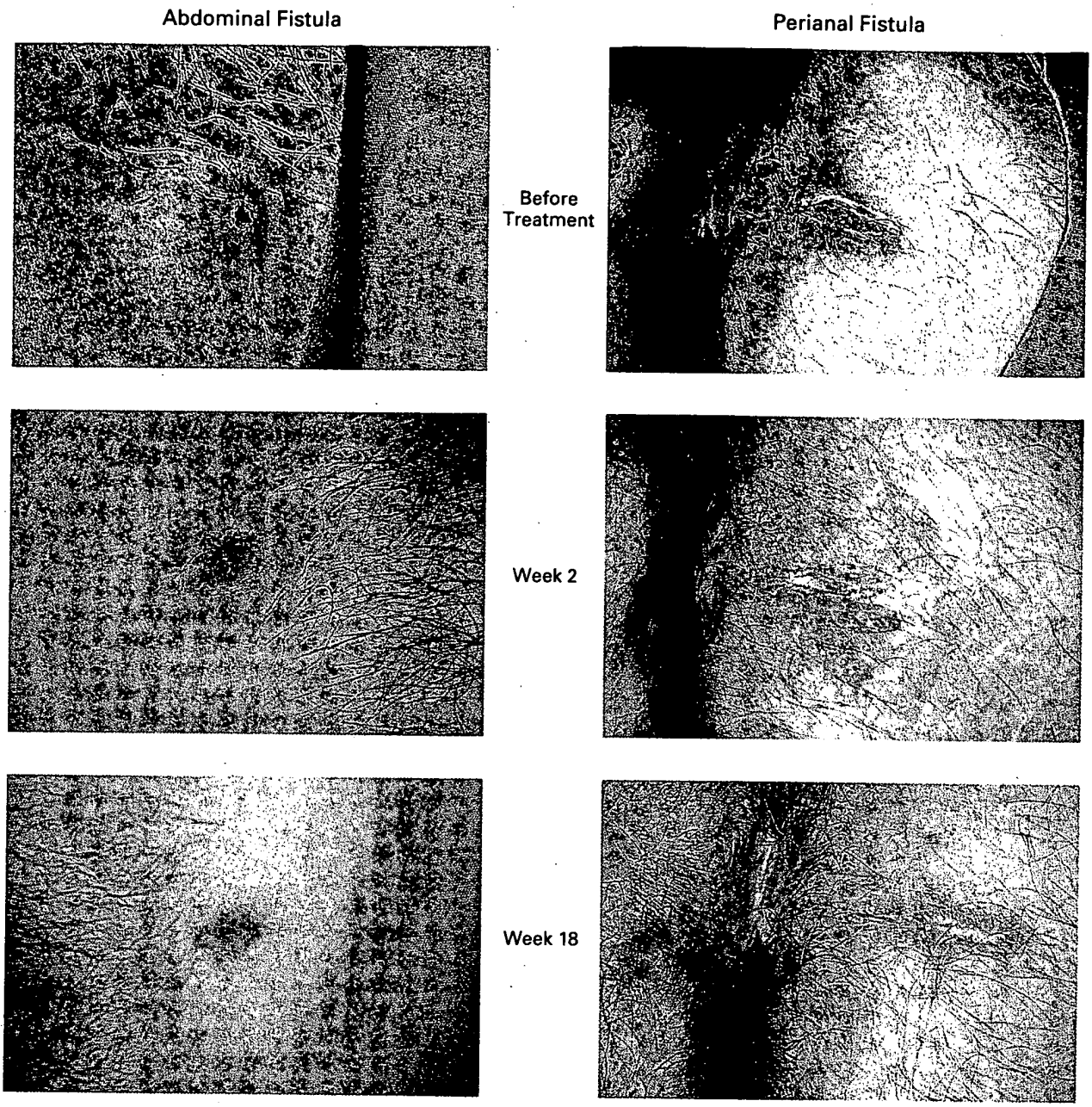

Figure 1. Closure of an Abdominal Fistula in a 60-Year-Old Man and a Perianal Fistula in a 42-Year-Old Man. Both patients received 5 mg of infliximab per kilogram.

same was true for patients with multiple fistulas (71 percent vs. 39 percent, P=0.03). In addition, infliximab was consistently beneficial regardless of concomitant therapy (e.g., corticosteroids, mercaptopurine or azathioprine, or antibiotics).

Safety

All 94 patients were evaluated for safety. The percentage of patients with adverse events was the same for the group assigned to receive 5 mg of infliximab per kilogram and that assigned to placebo (65 percent); there was a trend toward more adverse events among the patients assigned to receive 10 mg of infliximab per kilogram (84 percent, P=0.09). The most frequently reported adverse events among patients treated with infliximab were headache, abscess, upper respiratory tract infection, and fatigue (Table 4). One patient in the group receiving 10 mg of infliximab per kilogram discontinued treatment because of pneumonia, which developed 22 days after the second infusion. The symptoms resolved within a week with antibiotic treatment. Altogether, five patients had

TABLE 3. RESULTS OF TREATMENT ACCORDING TO SELECTED VARIABLES.

VARIABLE	PLACEBO		INFLIXIMAB		ODDS RATIO*	P VALUE
	NO. IN SUBGROUP	% WITH PRIMARY END POINT	NO. IN SUBGROUP	% WITH PRIMARY END POINT		
All patients	31	26	63	62	4.7	0.001
Sex						
Male	17	18	27	74	13.3	<0.001
Female	14	36	36	53	2.0	0.28
Area of involvement						
Ileum	3	0	11	73	—	—
Colon	9	33	17	53	2.3	0.35
Ileum and colon	19	26	35	65	5.1	0.01
No. of enterocutaneous fistulas						
1	13	8	29	52	12.9	0.02
>1	18	39	34	71	3.8	0.03
Dose of oral corticosteroids						
20 mg/day	5	20	6	67	8.0	0.14
<20 mg/day	6	17	15	53	5.7	0.15
None	20	30	42	64	4.2	0.01
Use of mercaptopurine or azathioprine						
Yes	9	44	29	59	1.8	0.46
No	22	18	34	65	8.3	0.001
Use of antibiotics						
Yes	11	27	17	65	4.9	0.06
No	20	25	46	61	4.7	0.01

*The odds ratio is for the odds of reaching the primary end point in the infliximab group as compared with the placebo group.

serious adverse events: four assigned to receive 10 mg of infliximab per kilogram and one assigned to receive 5 mg per kilogram. In the 10-mg group, these events were chest pain and pneumonia (in the patient who discontinued treatment), intestinal obstruction, abscess of the arm and leg (furunculosis), and anal abscess. In the patient in the 5-mg infliximab group, ureteral obstruction developed after the third infusion. In four of the patients receiving infliximab (6 percent), adverse events occurred during an infusion or within two hours after the end of the infusion, with some patients having multiple adverse reactions; these adverse events were mild dizziness in two patients, subfebrile temperature elevation in two, headache in one, and chest pain with flushing in two. There were no consistent differences in routine laboratory values between the infliximab and placebo groups. No deaths occurred during the study period.

Immunologic Results

Antibodies against double-stranded DNA were detected in eight patients treated with infliximab (13 percent); one patient remained positive for these antibodies at the last evaluation. None had symptoms suggestive of lupus erythematosus. Serum samples were collected both before and after treatment from 92 patients and assayed for human antichimeric antibodies. Three tested positive for human antichimeric antibodies, all at a titer of 1:10. Thirteen patients had measurable concentrations of infliximab in all post-treatment samples and therefore could not be evaluated. None of the adverse events in the patients who were positive for human antichimeric antibodies were suggestive of a sensitivity reaction.

TABLE 4. ADVERSE EVENTS THAT OCCURRED IN AT LEAST 10 PERCENT OF PATIENTS IN ANY TREATMENT GROUP.

EVENT	PLACEBO (N=31)	INFLIXIMAB		
		5 mg/kg (N=31)	10 mg/kg (N=32)	5 OR 10 mg/kg (N=63)
	number (percent)			
Headache	7 (23)	5 (16)	6 (19)	11 (17)
Abscess	1 (3)	2 (6)	5 (16)	7 (11)
Upper respiratory tract infection	2 (6)	1 (3)	5 (16)	6 (10)
Fatigue	2 (6)	2 (6)	4 (12)	6 (10)

DISCUSSION

Closure of fistulas is rare in patients with Crohn's disease who are receiving standard therapy, such as 5-aminosalicylates or corticosteroids. Several antibiotics have shown promise for the healing of fistulas

in Crohn's disease,[21-23] but their efficacy has not been established in controlled clinical trials. Immunomodulatory agents have been used to treat fistulas, with some success. In one uncontrolled study, fistulas closed in about one third of patients treated with methotrexate.[24] Small, uncontrolled studies have demonstrated that intravenous cyclosporine induces the closure of fistulas; however, patients relapsed when switched to oral cyclosporine.[4,25] A double-blind, placebo-controlled study[3] suggested that mercaptopurine was more effective than placebo in the treatment of fistulas in patients with Crohn's disease; however, the study had too few patients for the statistical significance of this finding to be assessed. In addition, approximately three months was required for a response to appear in patients treated with mercaptopurine.

In our study, we found a significant reduction in the number of draining fistulas in patients with Crohn's disease, as compared with the number at base line, after two or three infusions of infliximab at doses of 5 or 10 mg per kilogram. The effect of treatment with infliximab became evident rapidly — in about two weeks — and lasted for a median of three months; a complete response (defined as the absence of draining fistulas) occurred in 46 percent of patients treated with infliximab, as compared with 13 percent of the placebo group ($P=0.001$). The beneficial effect of infliximab did not appear to be dose-related; patients treated with 5 mg of infliximab per kilogram had a higher rate of response than those treated with 10 mg per kilogram (68 percent vs. 56 percent).

The frequency of adverse events was the same in the placebo group and the group assigned to 5 mg of infliximab per kilogram; there was a trend toward more adverse events in the group assigned to 10 mg of infliximab per kilogram. Eight patients treated with infliximab (13 percent) had low levels of antibodies against double-stranded DNA. In all but one, these antibodies disappeared by the end of the study. The clinical significance of these serologic findings is uncertain; none of the patients had symptoms suggestive of lupus erythematosus. In an earlier trial,[17] a duodenal lymphoma developed in one patient with a 30-year history of Crohn's disease. The association of this event with infliximab is uncertain, since the incidence of lymphoma is increased in chronic Crohn's disease.[26]

Some issues remain to be addressed regarding the use of infliximab in patients with Crohn's disease that is complicated by fistulas. These include the use of infliximab as an effective corticosteroid-sparing agent, the long-term toxicity of the regular or intermittent use of infliximab, and the best timing for the administration of infliximab. The majority of patients in this study had chronically active disease and had previously received several therapies, including immunosuppressive agents. The efficacy of a regimen based on infliximab as a first-line therapy to induce early closure of fistulas, with mercaptopurine or azathioprine reserved for long-term maintenance after fistulas have healed, needs further investigation.

In conclusion, we found that infliximab was efficacious in the treatment of enterocutaneous fistulas complicating Crohn's disease. Our results support the use of an initial dose of 5 mg per kilogram, with subsequent identical doses given two and six weeks later.

Dr. Hanauer, Dr. Podolsky, and Dr. Sands have served as paid consultants to Centocor. Dr. Present and Dr. Hanauer have received honorariums from Centocor for lectures. Dr. Mayer owns stock in Centocor.

APPENDIX

In addition to the authors, the following participated in the study: *Duke University Medical Center, Durham, N.C.* — J. Onken; *Methodist Hospital, Houston* — A.L. Buchman; *Thomas Jefferson University Hospital, Philadelphia* — A.J. DiMarino; *Saint Mark's Hospital, London* — M. Kamm; and *Leeds General Infirmary, Leeds, United Kingdom* — D.M. Chalmers.

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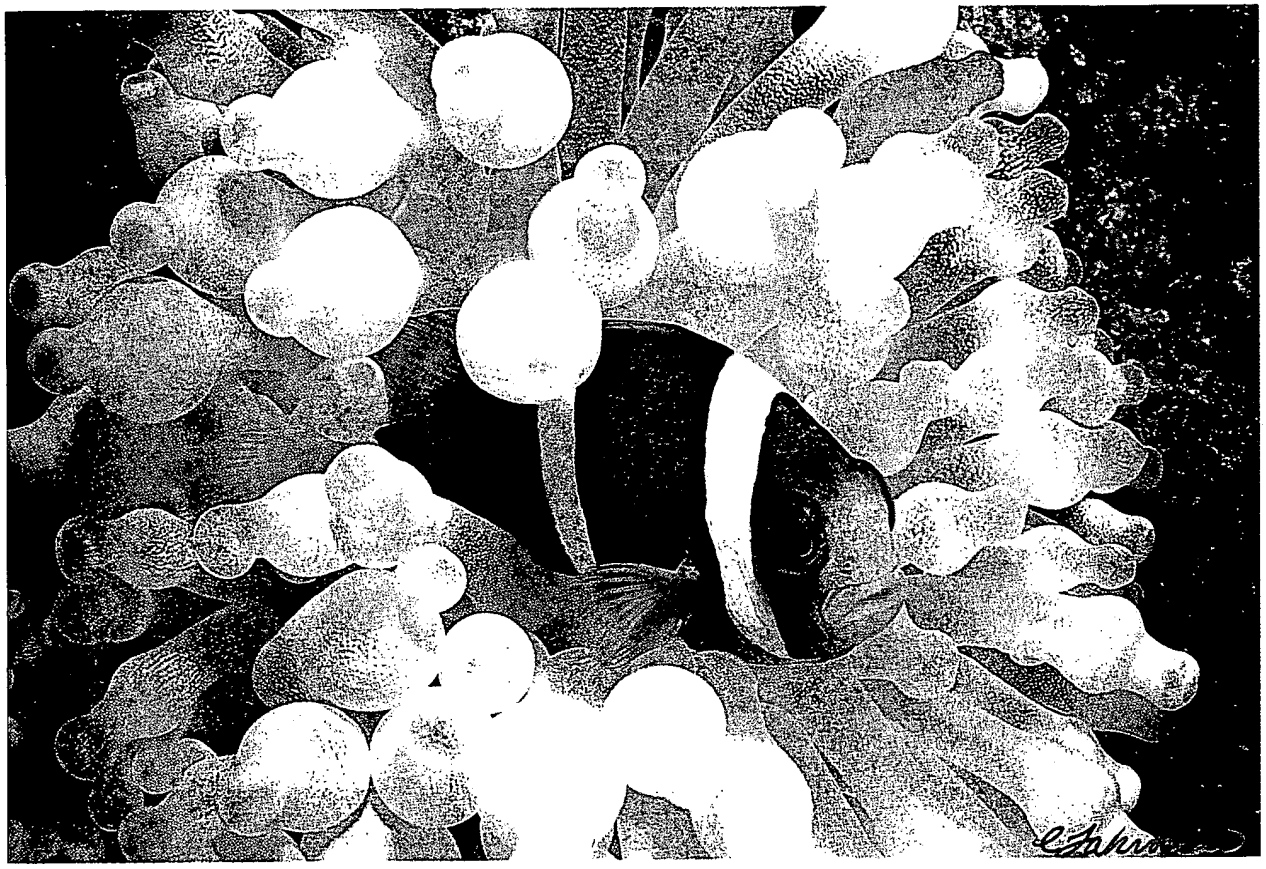

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ETANERCEPT IN CHILDREN WITH POLYARTICULAR JUVENILE RHEUMATOID ARTHRITIS

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ABSTRACT

Background We evaluated the safety and efficacy of etanercept, a soluble tumor necrosis factor receptor (p75):Fc fusion protein, in children with polyarticular juvenile rheumatoid arthritis who did not tolerate or had an inadequate response to methotrexate.

Methods Patients 4 to 17 years old received 0.4 mg of etanercept per kilogram of body weight subcutaneously twice weekly for up to three months in the initial, open-label part of a multicenter trial. Those who responded to treatment then entered a double-blind study and were randomly assigned to receive either placebo or etanercept for four months or until a flare of the disease occurred. A response was defined as an improvement of 30 percent or more in at least three of six indicators of disease activity, with no more than one indicator worsening by more than 30 percent.

Results At the end of the open-label study, 51 of the 69 patients (74 percent) had had responses to etanercept treatment. In the double-blind study, 21 of the 26 patients who received placebo (81 percent) withdrew because of disease flare, as compared with 7 of the 25 patients who received etanercept (28 percent) ($P=0.003$). The median time to disease flare with placebo was 28 days, as compared with more than 116 days with etanercept ($P<0.001$). In the double-blind study, there were no significant differences between the two treatment groups in the frequency of adverse events.

Conclusions Treatment with etanercept leads to significant improvement in patients with active polyarticular juvenile rheumatoid arthritis. Etanercept is well tolerated by pediatric patients. (N Engl J Med 2000;342:763-9.)

JUVENILE rheumatoid arthritis is the most common rheumatic condition in children.[1,2] In approximately one third of patients, the disease is controlled with nonsteroidal antiinflammatory drugs and an appropriate program of physical and occupational therapy. The remainder are candidates for more aggressive therapy with antirheumatic drugs.

Methotrexate was shown to have a therapeutic advantage over placebo, with an acceptable safety profile, in a randomized, controlled trial in children with juvenile rheumatoid arthritis who had polyarticular involvement (regardless of the type of onset).[3] Long-term studies showed that methotrexate is efficacious and well tolerated in most children with juvenile rheumatoid arthritis.[3-6] However, some patients do not have an adequate response to methotrexate, even at doses of up to 1 mg per kilogram of body weight per week.[7,8] The frequency and severity of side effects increase with higher doses of methotrexate, and the consequences of long-term use are not known. Exacerbation of disease during treatment with stable doses of methotrexate and the need to increase the methotrexate dose over time suggest that drug resistance to methotrexate may develop.[9]

Tumor necrosis factor (TNF) is a proinflammatory cytokine that has a complex role in the pathogenesis of rheumatoid arthritis.[10-17] TNF is elevated in both the serum and the synovial fluid of children with juvenile rheumatoid arthritis. Serum levels of soluble TNF receptor are elevated in patients with juvenile rheumatoid arthritis (all subtypes), and the level is correlated with disease activity.[18] In one study, tumor necrosis factor was detected in 45 percent of samples of synovial fluid from 44 children with juvenile rheumatoid arthritis (all subtypes).[19] Further evidence that TNF may amplify local inflammation and lead to joint destruction came from a study in which both TNF and lymphotoxin-α were detected in the majority of synovial-tissue samples from patients with juvenile rheumatoid arthritis.[20]

Etanercept (Enbrel, Immunex, Seattle), a genetically engineered fusion protein consisting of two identical chains of the recombinant extracellular human TNF-receptor p75 monomer fused with the Fc domain of human IgG1, effectively binds TNF and lymphotoxin-α and inhibits their activity.[21,22] Randomized, double-blind, placebo-controlled trials showed that etanercept treatment had significant clinical benefit with minimal toxicity in adults with active rheumatoid arthritis that did not respond to other disease-modifying drugs.[23-25] We conducted a randomized, multicenter, double-blind trial of etanercept for the

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treatment of polyarticular juvenile rheumatoid arthritis in children who did not tolerate or who had an inadequate response to methotrexate.

METHODS

Patients

Eligible patients were 4 to 17 years of age and had active polyarticular juvenile rheumatoid arthritis. During the first six months of the disease, some patients had had pauciarticular arthritis (four or fewer joints involved), some had had polyarticular arthritis (five or more joints involved), and some had had systemic arthritis (associated with spiking fever and rheumatoid rash). "Active" polyarticular disease was defined by the presence of five or more swollen joints and three or more joints with limitation of motion and pain, tenderness, or both. Before enrollment, patients had active disease despite treatment with nonsteroidal antiinflammatory drugs and with methotrexate at doses of at least 10 mg per square meter of body-surface area per week. The patients had normal or nearly normal platelet, white-cell, and neutrophil counts, hepatic aminotransferase levels, and results of renal-function tests. Pregnant and lactating patients were excluded, and girls with childbearing potential were required to use contraception throughout the study. Patients with major concurrent medical conditions were also ineligible.

Study Design

An independent review committee at each study site approved the protocol and amendments, and each patient's parent or legal guardian gave written informed consent before the start of the study. A safety monitoring board reviewed adverse events that occurred during the study. Methotrexate was discontinued 14 days and other disease-modifying antirheumatic drugs 28 days before receipt of etanercept. Intraarticular and soft-tissue corticosteroid injections were not permitted during or for one month before the trial. Stable doses of nonsteroidal antiinflammatory drugs, low doses of corticosteroids (≤0.2 mg of prednisone per kilogram per day, with a maximum of 10 mg per day), or both were permitted. Pain medications were allowed except during the 12 hours before a joint assessment.

Vials of study medication contained either 25 mg of lyophilized etanercept (for both parts of the study) or placebo (for the double-blind study). Before injection, study-site staff who were not involved in patient assessments reconstituted the contents with 1 ml of bacteriostatic water containing 0.9 percent benzyl alcohol.

All patients received 0.4 mg of etanercept per kilogram (maximum, 25 mg) subcutaneously twice weekly for up to three months in the open-label part of the trial. At the end of the third month, patients whose condition had improved according to the definition of Giannini et al.[26] were randomly assigned to receive either placebo or 0.4 mg of etanercept per kilogram subcutaneously twice weekly in the double-blind study (months 4 through 7) until disease flare occurred or four months elapsed, whichever was earlier. Efficacy was assessed according to the number of patients with disease flare after receipt of placebo or etanercept.

Physical examinations, measures of disease activity, and laboratory tests (hematologic analysis, serum chemical analysis, and urinalysis) were performed at screening and repeated on day 1 (before the administration of etanercept or placebo) and day 15 and at the end of each month during the study. Final safety assessments were made 30 days after the discontinuation of the study drug for patients who withdrew from the study or did not continue to the double-blind study, or at the patient's next scheduled visit if the patient withdrew from the study because of disease flare. Serum was obtained at screening and at the end of months 3 and 7 for testing for autoantibodies (antinuclear antibodies, antibodies to double-stranded DNA, IgG and IgM anticardiolipin antibodies, and antibodies to extractable nuclear antigens), and on day 1 before the administration of the study drug and at the end of months 3 and 7 for testing for antibodies to etanercept.

Definition of Improvement

The definition of improvement used to assess disease response employs a core set of six response variables: global assessment of the severity of disease by the physician, global assessment of overall well-being by the patient or parent, number of "active" joints (joints with swelling not due to deformity or joints with limitation of motion and with pain, tenderness, or both), number of joints with limitation of motion, functional ability, and erythrocyte sedimentation rate.[26,27] In this study, the fourth measure was modified to the "number of joints with limitation of motion and with pain, tenderness, or both" so as to eliminate counting joints with contractures that might not have improved during the short course of treatment.

To meet the definition of improvement at a scheduled visit or at the end of month 3, patients had to have a 30 percent improvement from base line in at least three of the six response variables. They could also have worsening of 30 percent or more in no more than one of the six response variables. Additional assessments of disease activity included the articular severity score,[28] duration of morning stiffness, degree of pain (on a visual-analogue scale), and C-reactive protein levels. Patients were also evaluated for 50 percent and 70 percent improvement (50 percent and 70 percent improvement in at least three of the six response variables and a worsening of 30 percent or more in no more than one of the six response variables).

The primary efficacy end point, which was evaluated in the double-blind study, was the number of patients with disease flare. The definition of disease flare created specifically for this pediatric trial was based on the change in the core set of response variables from the beginning of the double-blind study. Patients who met the criteria for disease flare had worsening of 30 percent or more in three of the six response variables and a minimum of two active joints. They also could have improvement of 30 percent or more in no more than one of the six response variables. Global assessments, if used to define flare, had to change by at least 2 units on a scale from 0 to 10.

Statistical Analysis

A blocked randomization scheme with stratification according to study center and number of active joints (≤2 vs. >2) at the end of month 3 (in the open-label study) was used to assign patients to placebo or etanercept in the double-blind study.

In the double-blind study, base-line and demographic characteristics were compared between treatment groups by the Wilcoxon rank-sum test and the likelihood-ratio chi-square test. Laboratory results were summarized separately from adverse events according to a modification of the National Cancer Institute Common Toxicity Criteria and the testing laboratory's normal ranges. Comparisons of shifts in laboratory values (to below normal, normal, or above normal) were made with use of the Stuart–Maxwell chi-square test.[29]

The percentages of patients with a response to therapy who had disease flare while receiving placebo or etanercept in the double-blind study were compared by Mantel–Haenszel methods.[30] Patients who withdrew early without disease flare were counted in the analysis with those who continued to have a response.

To evaluate any bias introduced by the withdrawal assumption in the primary analysis, an analysis of time to flare (by the log-rank test) was undertaken in which data on patients who withdrew without flare were censored at the time of withdrawal. The effect of base-line characteristics on flare rates was assessed by main-effects logistic regression. The percentages of patients with a response who continued to have a response after receiving etanercept or placebo in the double-blind study were compared by Mantel–Haenszel methods. All tests were two-sided, with a significance level of 0.05.

In all summaries of measures of disease activity, a last-observation-carried-forward approach was used for missing data or visits and for patients who withdrew early.

RESULTS

Base-Line Characteristics

The base-line demographic and disease characteristics of the study patients are summarized in Table 1.

TABLE 1. DEMOGRAPHIC CHARACTERISTICS AND DISEASE HISTORY.*

CHARACTERISTIC	OPEN-LABEL STUDY (N=69)	DOUBLE-BLIND STUDY		
		TOTAL (N=51)	PLACEBO (N=26)	ETANERCEPT (N=25)
Mean age — yr	10.5	10.6	12.2	8.9
Age group — no. (%)				
4–8 yr	25 (36)	18 (35)	5 (19)	13 (52)
9–12 yr	14 (20)	9 (18)	4 (15)	5 (20)
13–17 yr	30 (43)	24 (47)	17 (65)	7 (28)
Sex — no. (%)				
Female	43 (62)	34 (67)	15 (58)	19 (76)
Male	26 (38)	17 (33)	11 (42)	6 (24)
Race or ethnic group — no. (%)				
White	52 (75)	37 (73)	23 (88)	14 (56)
Black	6 (9)	4 (8)	1 (4)	3 (12)
Hispanic	9 (13)	8 (16)	2 (8)	6 (24)
Other	2 (3)	2 (4)	0	2 (8)
Type of onset of JRA — no. (%)				
Pauciarticular	7 (10)	3 (6)	1 (4)	2 (8)
Polyarticular	40 (58)	31 (61)	17 (65)	14 (56)
Systemic	22 (32)	17 (33)	8 (31)	9 (36)
Mean duration of JRA — yr	5.9	5.8	6.4	5.3
Positive for rheumatoid factor — no. (%)	15 (22)	12 (24)	8 (31)	4 (16)
Previous methotrexate therapy — no. (%)	69 (100)	51 (100)	26 (100)	25 (100)
DMARDs at washout — no. (%)	51 (74)	35 (69)	19 (73)	16 (64)
Methotrexate	50 (72)	34 (67)	18 (69)	16 (64)
Hydroxychloroquine	13 (19)	9 (18)	7 (27)	2 (8)
Concomitant therapy at washout — no. (%)				
Corticosteroids	25 (36)	19 (37)	13 (50)	6 (24)
NSAIDs	66 (96)	49 (96)	24 (92)	25 (100)
Mean dose of corticosteroids — mg/day	5.6	5.8	5.5	6.5

*Percentages may not total 100, because of rounding. JRA denotes juvenile rheumatoid arthritis, DMARDs disease-modifiying antirheumatic drugs, and NSAIDs nonsteroidal antiinflammatory drugs.

Forty-three female and 26 male patients were enrolled in the open-label study; of these, 34 female and 17 male patients continued to the double-blind study. At enrollment, the mean age was 10.5 years (range, 4 to 17) and the mean duration of juvenile rheumatoid arthritis was 5.9 years. The groups were well balanced in the double-blind study, except for age group and race ($P<0.02$) and corticosteroid use at base line ($P=0.05$). The unequal randomization did not affect the study results.

Sixty-four of the 69 patients enrolled in part 1 (93 percent) completed treatment. Early discontinuations were due to an adverse event in one patient who had urticaria with the first dose of etanercept, refusal of treatment by two patients, and lack of response in two patients. Of the 25 patients in the etanercept group in the double-blind trial, 19 (76 percent) completed treatment and 6 withdrew because of disease flare. Seven of the 26 patients in the placebo group (27 percent) completed the study; 1 withdrew because of parental refusal to allow continuation, and 18 withdrew because of disease flare.

Disease Response (Open-Label Study)

Fifty-one of the 69 patients enrolled in the open-label study (74 percent) met the definition of improvement at the end of that study. Considerable improvements in all measures of disease activity were seen with etanercept, and improvement was noted in patients as early as the first evaluation, two weeks after the beginning of treatment (Table 2). Forty-four of the 69 patients (64 percent) met the definition of 50 percent improvement, and 25 (36 percent) met the definition of 70 percent improvement at the end of the open-label study (Fig. 1).

Efficacy (Double-Blind Study)

Disease Flare

In the double-blind study, significantly more patients who received placebo (21 of 26 [81 percent]) than patients who received etanercept (7 of 25 [28 percent], $P=0.003$) had disease flare. The rates of flare remained consistently and significantly lower in the etanercept group ($P<0.001$) after adjustment for

TABLE 2. MEASURES OF DISEASE ACTIVITY AND IMPROVEMENT FROM BASE LINE.*

MEASURE	OPEN-LABEL STUDY (N=69)					DOUBLE-BLIND STUDY, PLACEBO (N=26)			DOUBLE-BLIND STUDY, ETANERCEPT (N=25)		
	BASE LINE	MO 1	MO 2	MO 3	% IMPROVEMENT†	BASE LINE	MO 3	MO 7	BASE LINE	MO 3	MO 7
Juvenile rheumatoid arthritis core set criteria											
Total no. of active joints‡	28	22	15	13	56	27.0	7.5	13.0	32.0	13.0	7.0
No. of joints with limitation of motion and with pain, tenderness, or both‡	10	4	3	2	79	6.5	1.0	4.5	8.0	2.0	1.0
Physician's global assessment of disease severity§	7	3	3	2	60	6	1	5	7	2	2
Patient's or parent's global assessment of overall well-being§	5	3	3	2	50	5	1	5	5	2	3
Score on Childhood Health Assessment Questionnaire¶	1.4	1.0	0.9	0.9	37	1.3	0.4	1.2	1.6	0.9	0.8
Erythrocyte sedimentation rate‖	35	18	20	20	50	27	12	30	41	15	18
Additional assessments											
Articular severity score**	88	60	47	45	50	84	36	66	90	35	38
Duration of stiffness (min)	45	15	15	15	75	60	5	38	45	15	5
Pain (on a visual-analogue scale)††	3.6	2.1	1.3	1.4	63	3.5	0.3	3.5	3.5	1.3	1.5
C-reactive protein (mg/dl)‡‡	3.5	0.9	1.1	0.8	60	1.8	0.3	3.0	3.5	0.2	0.4
Other‡											
No. of swollen joints	25	16	11	9	58	22.5	6.0	11.0	27.0	12.0	4.0
No. of joints with limitation of motion	23	20	18	15	23	23	17	22	24	12	9

*All values are medians. A last-observation-carried-forward approach was used for missing data and visits and for early termination.

†The percent improvement between base line and month 3 is shown. Patients who had values of zero at base line were omitted from calculations of percent change. Sixty-three patients were included in the analysis of duration of stiffness; 65 in the analysis of joints with both limitation of motion and pain, tenderness, or both, and pain (on a visual-analogue scale); and 67 in the analysis of patient's or parent's global assessment and the score on the Childhood Health Assessment Questionnaire.

‡Seventy-three joints were evaluated for the total active-joint count; 71 for limitation of motion with pain, tenderness, or both; 66 for swollen joints; and 71 for limitation of motion.

§Scores could range from 0 (best) to 10 (worst).

¶Scores could range from 0 (best) to 3 (worst).

‖The normal ranges are 1 to 30 mm per hour for females and 1 to 13 mm per hour for males.

**Scores could range from 0 (best) to 962 (worst).

††Scores could range from 0 cm (best) to 10 cm (worst).

‡‡The normal range is 0 to 0.79 mg per deciliter.

the effects of base-line characteristics (Table 3). With the exception of corticosteroid use at base line ($P=0.05$), none of the base-line characteristics were significant predictors of flare rates ($P>0.15$) (Table 3).

The median time to flare was more than 116 days for patients who received etanercept and 28 days for patients who received placebo ($P<0.001$) (Fig. 2). Because 13 of 25 patients were still receiving etanercept at the end of the study (day 116) without disease flare, the median time to flare was greater than 116 days.

Disease Response at End of Study

The definition of improvement was based on changes from base-line values, whereas disease flare was based on changes from values at the time of randomization to either etanercept or placebo in the double-blind study. Depending on the magnitude of a patient's response in the open-label study, response to treatment and presence of disease flare were not mutually exclusive outcomes. For example, if a patient had 28 active joints at base line but only 2 active joints at the time of randomization, a change to 3 active joints would be considered a flare (at least 30 percent worse than the condition at the time of randomization) but would also still be considered improvement (at least 30 percent improved from base line). At the end of the seven-month study, 20 of the 25 patients who received etanercept in the double-blind study (80 percent) still met the definition of improvement, as compared with 9 of the 26 patients who received placebo (35 percent, $P<0.01$).

At the end of the study, 72 percent of the patients who received etanercept (18 patients) and 23 percent of those who received placebo (6 patients) met the definition of 50 percent improvement. Forty-four percent of the patients who received etanercept (11 patients) and 19 percent of those who received placebo (5 patients) met the definition of 70 percent improvement. Measures of disease activity continued to improve in patients who received etanercept in the double-blind study, whereas disease activity increased in those who received placebo (Table 2).

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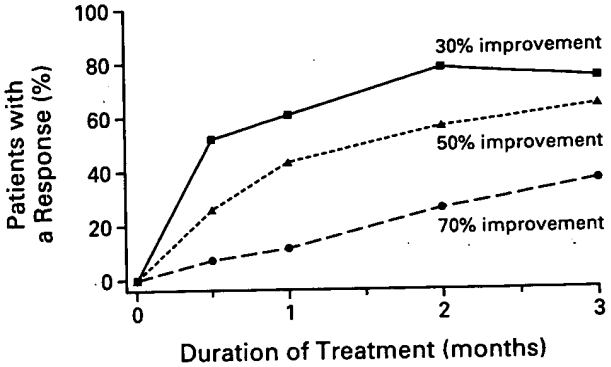

Figure 1. Incidence of 30, 50, and 70 Percent Improvement in the 69 Patients Who Received Etanercept in the Open-Label Study.

At the end of the open-label study, 51 (74 percent) of the patients had a 30 percent improvement, 44 (64 percent) had a 50 percent improvement, and 25 (36 percent) had a 70 percent improvement, as compared with base line.

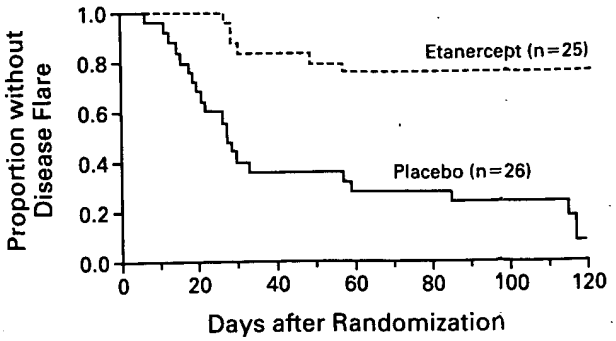

Figure 2. Kaplan–Meier Analysis of the Time to Disease Flare.

The median time to disease flare was significantly shorter among the patients who received placebo (28 days) than among those who received etanercept (>116 days, P<0.001) in the double-blind study.

TABLE 3. INCIDENCE OF DISEASE FLARE IN THE DOUBLE-BLIND STUDY ACCORDING TO THE BASE-LINE CHARACTERISTICS OF THE PATIENTS.

VARIABLE*	PLACEBO	ETANERCEPT
	no./total no. (%)	
Total with disease flare	21/26 (81)	7/25 (28)
Age group		
4–8 yr	4/5 (80)	3/13 (23)
9–12 yr	4/4 (100)	1/5 (20)
13–17 yr	13/17 (76)	3/7 (43)
Sex		
Female	14/15 (93)	5/19 (26)
Male	7/11 (64)	2/6 (33)
Race or ethnic group		
White	18/23 (78)	4/14 (29)
Black	1/1 (100)	1/3 (33)
Hispanic	2/2 (100)	1/6 (17)
Other	0	1/2 (50)
Rheumatoid factor		
Positive	8/8 (100)	0/4 (0)
Negative	13/18 (72)	7/21 (33)
Type of juvenile rheumatoid arthritis at onset		
Pauciarticular	1/1 (100)	0/2 (0)
Polyarticular	13/17 (76)	3/14 (21)
Systemic	7/8 (88)	4/9 (44)
Corticosteroid use at base line†		
Yes	12/13 (92)	3/6 (50)
No	9/13 (69)	4/19 (21)

*For each of the variables in this table, the difference in flare rates between the placebo and etanercept groups continued to be statistically significant (P<0.001) when the effect of the variable was controlled for in a logistic-regression model.

†Corticosteroid use at base line was the only variable found to be predictive of flare rates in the placebo and etanercept groups (P=0.05) in a logistic-regression model. For the other variables, the P value was greater than 0.15.

Health Assessment Questionnaire[31,32] (a measure of the patient's physical functional ability) began to improve at the first evaluation two weeks after the beginning of etanercept treatment. At the end of the open-label study, a 37 percent median improvement in scores was seen for all patients. In the double-blind study, a 54 percent median improvement in scores from base line was seen in patients who continued to receive etanercept, as compared with no change from base line in patients who received placebo (P=0.01).

In a significant proportion of patients, there were shifts from elevated values at base line to normal values of C-reactive protein, erythrocyte sedimentation rate, and white-cell and platelet counts after treatment with etanercept in the open-label study (P<0.03 for each variable). In the double-blind study as compared with the end of the open-label study, a significant proportion of patients who received placebo had shifts from normal levels of C-reactive protein and erythrocyte sedimentation rates to above-normal values (P≤ 0.03 for each variable).

Safety

Etanercept was safe and well tolerated. There were no deaths. One patient withdrew because of urticaria after the first dose of etanercept; the urticaria responded to oral antihistamines. This patient later received commercially available etanercept without recurrence of urticaria. Two patients who received etanercept were hospitalized for serious adverse events (one for depression and a personality disorder and one for gastroenteritis–flu syndrome). All other adverse events were of mild-to-moderate intensity.

In the open-label study, the most common adverse events were injection-site reactions (39 percent of patients), upper respiratory tract infections (35 percent), headache (20 percent), rhinitis (16 percent), abdominal pain (16 percent), vomiting (14 percent), pharyn-

gitis (14 percent), nausea (12 percent), gastrointestinal infection (12 percent), and rash (10 percent).

In the double-blind study, there were no significant differences in the frequencies of adverse events between patients who received etanercept and those who received placebo. Injection-site reactions occurred in one patient in each of the treatment groups in the double-blind study. There were no laboratory abnormalities requiring urgent treatment in the etanercept group. No patient had persistent elevations in autoantibodies or had signs or symptoms of another autoimmune disease. Two patients tested positive for nonneutralizing antibody to etanercept. Fifty-nine of the 68 eligible patients in the study chose to continue treatment in an open-label, extended-treatment study.

DISCUSSION

The choice of a second-line agent for the treatment of juvenile rheumatoid arthritis has become more difficult because placebo-controlled trials and long-term prospective studies in children with juvenile rheumatoid arthritis have shown a lack of efficacy of agents commonly used in adults.[33-37] Methotrexate is not efficacious or well tolerated in some patients with juvenile rheumatoid arthritis, and higher doses of methotrexate may be associated with greater toxicity.[7]

The design of this double-blind, placebo-controlled trial was sensitive to the problems of the population of patients with severe juvenile rheumatoid arthritis, for whom few treatment options are available. The study design allowed all patients to try the new treatment; only those who had a response to treatment were enrolled in the randomized portion of the trial. In addition, the definition of disease flare did not require the disease to become as severe as at base line. The protocol allowed patients to discontinue the study immediately after disease flare and be treated with etanercept in an open-label, long-term program. With the definition of disease flare used in this study, we effectively demonstrated differences in flare rates between the treatment groups.

The dose of etanercept used in this study (0.4 mg per kilogram) provided a favorable risk–benefit profile in children with polyarticular juvenile rheumatoid arthritis. Adverse events were of the types and intensity seen in a general pediatric population. There were no life-threatening adverse events, and the events were self-limited. A continued response was documented in patients who received etanercept in the double-blind study. At the end of the study, 80 percent of the patients who received etanercept for seven months met the definition of 30 percent improvement, as compared with 35 percent of the patients who received etanercept for three months and placebo for up to four months.

The results of this study confirm that TNF, lymphotoxin-α, or both have a role in juvenile rheumatoid arthritis, and that inhibition of these substances is a valid therapeutic intervention. Etanercept was effective in pediatric patients with severe polyarticular juvenile rheumatoid arthritis (regardless of the type of onset) who did not tolerate or have an adequate response to methotrexate. The significant clinical response supports the use of etanercept in children with juvenile rheumatoid arthritis.

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IMAGES IN CLINICAL MEDICINE

The *Journal* has a large backlog of Images in Clinical Medicine that have been accepted for publication. Therefore, we will not consider new submissions in 2000. This decision will be reevaluated in December.

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Etanercept in the treatment of psoriatic arthritis and psoriasis: a randomised trial

Philip J Mease, Bernard S Goffe, James Metz, Ann VanderStoep, Barbara Finck, Daniel J Burge

Summary

Background Etanercept, a tumour-necrosis-factor inhibitor, has shown efficacy in the treatment of rheumatoid arthritis. Psoriatic arthritis and psoriasis are disease states in which tumour necrosis factor, a proinflammatory cytokine, is present in increased concentrations in joints and in the skin. Therefore, psoriatic arthritis and psoriasis may be appropriate therapeutic targets for etanercept.

Methods This randomised, double-blind, placebo-controlled, 12 week study assessed the efficacy and safety of etanercept (25 mg twice-weekly subcutaneous injections) or placebo in 60 patients with psoriatic arthritis and psoriasis. Psoriatic arthritis endpoints included the proportion of patients who met the Psoriatic Arthritis Response Criteria (PsARC) and who met the American College of Rheumatology preliminary criteria for improvement (ACR20). Psoriasis endpoints included improvement in the psoriasis area and severity index (PASI) and improvement in prospectively-identified individual target lesions.

Findings In this 12 week study, 26 (87%) of etanercept-treated patients met the PsARC, compared with seven (23%) of placebo-controlled patients. The ARC20 was achieved by 22 (73%) of etanercept-treated patients compared with four (13%) of placebo-treated patients. Of the 19 patients in each treatment group who could be assessed for psoriasis (≥3% body surface area), five (26%) of etanercept-treated patients achieved a 75% improvement in the PASI, compared with none of the placebo-treated patients (p=0·015). The median PASI improvement was 46% in etanercept-treated patients versus 9% in placebo-treated patients; similarly, median target lesion improvements were 50% and 0, respectively. Etanercept was well tolerated.

Interpretation Etanercept offers patients with psoriatic arthritis and psoriasis a new therapeutic option for control of their disease.

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Introduction

Commonly used topical therapies for skin lesions in psoriasis include moisturisers, corticosteroids, tar, anthralin, vitamin D analogues and retinoids, and ultraviolet-light therapy. When these therapies are inadequate, systemic therapies such as psoralen-ultraviolet-light treatment, methotrexate, ciclosporin, and acitretin may be used.[1–2] However, toxicities often limit the usefulness of these therapies.[3–5]

Psoriatic arthritis affects from 5% to 7% of patients with psoriasis.[6] Although psoriatic arthritis may present in a symmetric polyarticular form similar to rheumatoid arthritis, unique features include the potential for asymmetric, oligoarticular, axial and/or distal interphalangeal joint involvement, dactylitis, and enthesial inflammation.[7] Like rheumatoid arthritis, this disorder results in joint damage, disability, and increased mortality.[8–11]

Therapy for psoriatic arthritis has largely been derived from clinical experience in rheumatoid arthritis, without corroborating evidence from studies in patients with psoriatic arthritis.[12–14] The response to therapy is often unsatisfactory. The few controlled trials assessing patients with psoriatic arthritis have not shown consistent efficacy.[15–20]

Etanercept functions by inhibiting tumour necrosis factor, a proinflammatory cytokine that is involved in many inflammatory disorders, including both psoriatic arthritis and psoriasis. Tumour necrosis factor has been shown to be increased in synovial fluid and synovium in patients with psoriatic arthritis and in the skin of psoriatic lesions.[21–24] Tumour-necrosis-factor inhibition with etanercept has previously been shown to diminish the activity of rheumatoid arthritis.[25] Our study was undertaken to assess the benefit of etanercept in psoriatic arthritis and psoriasis.

Methods

Patients

Eligible patients were adults between 18 and 70 years who had active psoriatic arthritis (defined as ≥3 swollen joints and ≥3 tender or painful joints) at the time of study enrolment. Patients must have had an inadequate response to non-steroidal anti-inflammatory drugs and were thought candidates for immunomodulatory therapy. Patients taking methotrexate (≤25 mg/week) were allowed to continue methotrexate if the dose was stable for 4 weeks before study start and remained stable throughout the study. Other disease-modifying anti-rheumatic drugs (DMARDs) were discontinued at least 2 weeks before beginning the study drug and were not allowed during the study. Corticosteroids were allowed if the dose was less than or equal to 10 mg/day of prednisone, stable for at least 2 weeks before the first dose of study drug, and maintained at a constant dose throughout the study. Patients with evidence of skin

conditions other than psoriasis (such as eczema) were not allowed to enter the study. Topical therapies and oral retinoids for psoriasis were discontinued at least 2 weeks before the baseline evaluation and phototherapy was discontinued at least 4 weeks before treatment. All patients were required to have hepatic transaminase concentrations no greater than twice the upper limit of normal, haemoglobin 85 g/L or higher, platelet count 125000 per mL or more, and serum creatinine 152·4 mmol/L or below.

Study protocol

The protocol was approved by the human research committee for the centre, and all patients gave written informed consent before entering the study. Clinical and laboratory assessments done at screening, baseline, and 12 weeks, and consisted of physical examination, vital signs, measures of disease activity (arthritis and psoriasis), concomitant medications, laboratory studies (haematology, serum chemistry, urinalysis), and monitoring of adverse events. Additionally, arthritis disease-activity measures, adverse events, and concomitant medications were monitored at 4 weeks, 8 weeks, and 30 days after the last dose of the study drug (for those patients who withdrew prematurely). The measures of arthritis disease activity included assessments of 78 joints for tenderness and 76 joints for swelling (graded 0–3), patient's and physician's global assessments (on a 0–5 Likert scale), patient's assessment of pain, patient's assessment of disability as indicated by responses on the Health Assessment Questionnaire (HAQ), erythrocyte sedimentation rate, and serum concentration of C-reactive protein. Only patients with plaque psoriasis affecting greater than or equal to 3% of body surface area were assessed for skin disease. The measures of psoriasis activity included the psoriasis area and severity index (PASI)[26] and assessments of prospectively identified lesions (target lesions, assessed for plaque elevation, scaling, and erythema). Adverse events and abnormal laboratory values were graded on a scale derived from the common toxicity criteria of the National Cancer Institute.

Treatment

Patients with psoriatic arthritis were randomised to receive either placebo or etanercept (Enbrel) at a dose of 25 mg twice weekly by subcutaneous administration for 12 weeks. Patients who continued on methotrexate were randomised separately. A block randomisation was used: within each group of four patients enrolled, two were assigned at random to the placebo group and two to the etanercept group. Etanercept was supplied as a sterile, lyophilised powder in vials containing 25 mg etanercept, 40 mg mannitol, 10 mg sucrose, and 1·2 mg tromethamine per vial. Placebo was identically supplied and formulated except that it contained no etanercept. Each vial was reconstituted with 1 mL bacteriostatic water for injection.

Study endpoints

The primary endpoint with respect to efficacy in psoriatic arthritis was the proportion of patients who met the Psoriatic Arthritis Response Criteria (PsARC, adapted from Clegg and colleagues)[15] at 12 weeks. This composite measure requires improvement in two factors

Characteristic	Placebo (n=30)	Etanercept (n=30)
Median (range) age (years)	43·5 (24·0–63·0)	46·0 (30·0–70·0)
Male	18 (60%)	16 (53%)
White	25 (83%)	27 (90%)
Median (range) weight (kg)	81·4 (60·3–131·5)	90·7 (58·0–141·0)
Duration psoriatic arthritis (years, median [range])	9·5 (1·0–30·0)	9·0 (1·0–31·0)
Duration psoriasis (years, median) [range]	17·5 (2·0–43·0)	19·0 (4·0–53·0)
Number previous DMARDS (median [range])	2·0 (1·0–5·0)	1·5 (0–4·0)
Concomitant therapy during study		
Corticosteroids	12 (40%)	6 (20%)
NSAIDs	23((77%)	20 (67%)
Methotrexate	14 (47%)	14 (47%
Patients evaluable* for psoriasis endpoints (median [range])	**(n=19)**	**(n=19)**
Duration of psoriasis in years	2·0 (5·0–43·0)	20·0 (4·0–53·0)
Baseline PASI score	6·0 1·5–17·7)	10·1 (2·3–30·0)
Target lesion assessment	6·0 (3·0–8·0)	6·0 (3·0–9·0)

*≥3% body surface area involvement.

Table 1: **Demographic and clinical characteristics**

(with at least one being a joint score), with worsening in none, of the following four factors: patient and physician global assessments (improvement defined as decrease by ≥1 unit; worsening defined as increase by ≥1 unit); and tender and swollen joint scores (the sums of all joints scored; improvement defined as decrease by ≥30%; worsening defined as increase by ≥30%). A secondary endpoint for the assessment of psoriatic arthritis was the proportion of patients meeting the American College of Rheumatology preliminary criteria for improvement (ACR20; designed for assessment of rheumatoid arthritis)[27] at 12 weeks, which requires at least 20% reductions in tender and swollen joint counts and in at least three of the following: patient's assessment of pain, patient's global assessment, physician's global assessment, patient's assessment of disability, and acute phase reactant (C-reactive protein). ACR50 and ACR70 were also assessed (defined in a similar manner as ACR20, but with improvement of at least 50% and 70% in the individual measures, respectively). Individual measures of arthritis disease activity were also assessed.

The primary endpoint with respect to efficacy in psoriasis was the proportion of patients achieving a 75% improvement in psoriasis activity from baseline to 12

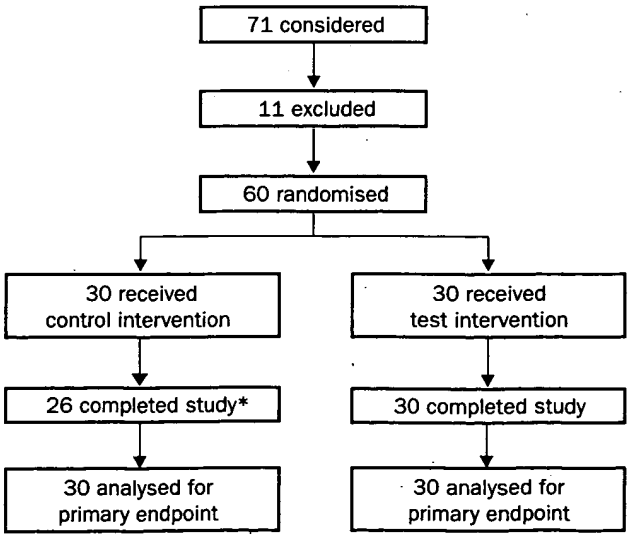

Figure 1: **Trial profile**

*Last observation was carried forward for efficacy analyses.

	Etanercept (n=30)	Placebo (n=30)	Difference	95% CI	p
Primary endpoint—achieved PsARC					
4 weeks	23 (77)	4 (13)	63%	44–83	<0·0001
8 weeks	25 (83)	8 (27)	57%	36–77	<0·0001
12 weeks	26 (87)	7 (23)	63%	44–83	<0·0001
Secondary endpoint—achieved at 12 weeks					
ACR20	22 (73)	4 (13)	60%	40–80	<0·0001
ACR50	15 (50)	1 (3)	47%	28–66	0·0001
ACR70	4 (13)	0	13%	1–26	0·0403

Table 2: **Psoriatic arthritis endpoints—number achieving PsARC and ACR20, ACR50, and ACR70, baseline to week 12**

weeks as measured by the PASI.[26] Additional analyses were done of the percentage change in PASI scores and improvement in the target psoriasis lesions.

Statistical analyses

On the assumption that response rates of 30% in the placebo group and 75% in the etanercept group, the sample size of 30 patients per treatment group gave over 80% power to detect a significant difference between treatments in the primary endpoint, by a two-sided α=0·05 level test. Proportions of patients responding were compared between treatment groups with the Mantel-Haenszel χ^2 test, adjusted for the stratification variable, methotrexate use. Continuous efficacy variables (percentage change from baseline) were ranked and analysed by a general linear model with factors of treatment, methotrexate use, and their interaction. The frequency of adverse events was compared between treatment groups with Fisher's exact test. The Breslow-Day test was used to test for heterogeneity of relative response between methotrexate use strata. All tests were two-sided. The last available observation was used for patients who discontinued treatment before the end of study.

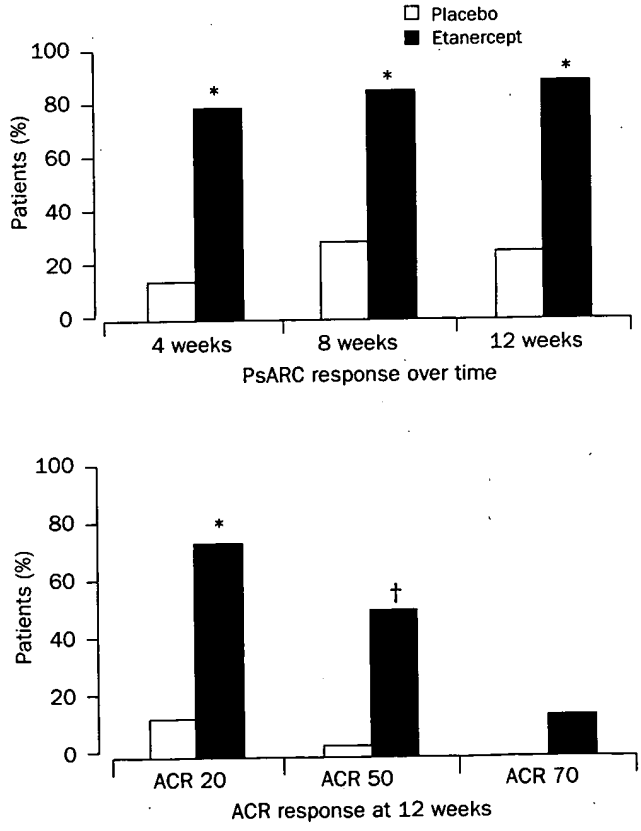

Figure 2: **Percentage of patients with PsARC responses over time and with ARC20, ARC50, and ARC70 responses at 12 weeks**
*p<0·0001. †p=0·0001.

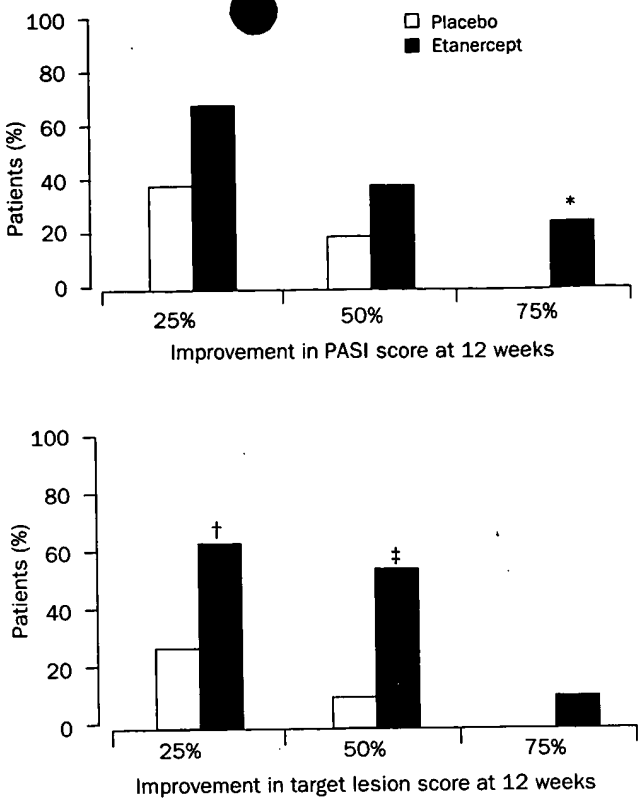

Figure 3: **Percentage of patients with at least 25%, 50%, and 75% improvements in PASI and target lesion scores**
*p=0·0154. †p=0·0098. ‡p=0·0006.

Results

Table 1 shows baseline demographic and clinical characteristics. 60 patients were randomised, 30 in each treatment arm (figure 1). The median age was 45 years (range 24–70) and the median duration of psoriatic arthritis was 10 years (1–31). The median duration of psoriasis was 18 years (2–53) for all patients in the study and was 20 years (4–53) for the 38 patients with evaluable psoriasis (19 patients in each group). The median active joint counts at baseline were 20 tender and 14 swollen joints. The groups were well balanced in all characteristics except that twice as many patients in the placebo group were receiving corticosteroids than in the etanercept group, and in the groups evaluable for psoriasis, the placebo group had lower baseline PASI scores. All patients in the etanercept group completed the 12 week study; four patients in the placebo group discontinued the study prematurely, three for lack of efficacy or refusal and one was lost to follow-up.

Efficacy of etanercept in psoriatic arthritis

The etanercept group had statistically better outcomes for all clinical endpoints (table 2, figure 2). The primary endpoint for psoriatic arthritis, the number of patients who met the PsARC at 12 weeks, was achieved by 26 (87%) etanercept-treated patients compared with seven (23%) placebo-treated patients (p<0·0001). The response was significantly greater at all measured time points in patients who received etanercept relative to those receiving placebo. The ACR response rates were also significantly higher in the etanercept-treated group.

	Placebo (n=30)	Etanercept (n=30)
Tender joint count*		
Baseline	19·0 (10, 39)	22·5 (11, 32)
12 weeks	22·5 (11, 47)	6·0 (1, 11)
Swollen joint count†		
Baseline	14·7 (7, 24)	14·0 (8, 23)
12 weeks	11·0 (5, 28)	3·0 (1, 8)
HAQ‡		
Baseline	1·2 (0·8, 1·6)	1·3 (0·9, 1·6)
12 weeks	1·1 (0·5, 1·5)	0·1 (0, 1)
ESR§		
Baseline	16 (9, 29)	22 (9, 34)
12 weeks	18 (6, 40)	5 (3, 12)
CRP‖		
Baseline	12 (8, 22)	14 (7, 28)
12 weeks	14 (4, 23)	4 (3, 11)

p<0·001 for all treatment comparisons.
*Scale 0–78; †Scale 0–76; ‡0=best, 3=worst; §Normal range: 1–13 mm/h for men; 1–30 mm/h for women; ‖Normal range: 0–0·78 mg/L.
ESR=erythrocyte sedimentation rate. CRP=C-reactive protein.

Table 3: **Secondary psoriatic arthritis endpoint—median (25th and 75th percentiles) values of disease activity, baseline, and 12 weeks**

At 12 weeks, the ACR20 was achieved by 22 (73%) etanercept-treated patients compared with four (13%) placebo-treated patients (p<0·0001).

At 12 weeks, the etanercept group showed significant improvement in all measures of disease activity compared with the placebo group (p≤0·0002). The median percentage improvements in tender and swollen joints counts for etanercept-treated patients were 75% and 72%, respectively, compared with 5% worsening and 19% improvement in placebo-treated patients. Disability, as assessed by responses on the Health Assessment Questionnaire, was also significantly more improved in the etanercept group than in the placebo group, with improvements of 83% and 3% from baseline, respectively (p<0·0001, table 3).

Table 4 shows the results of a comparison of patients treated with etanercept and placebo who achieved 100% improvement in individual disease-activity measures at 12 weeks. Ten (34%) patients in the etanercept group achieved disability index scores of 0 (no disability) at 12 weeks, compared with only one (3%) patient in the placebo group. In addition, seven (23%) of patients in the etanercept group had no swollen joints and four (13%) had no tender joints, compared with none in both cases in the placebo group.

Characteristic	Placebo (n=30)	Etanercept (n=30)
Factor (actual value)*		
Tender joint count (none)	0	4 (13)
Swollen joint count (none)	0	7 (23)
Total tender/swollen joint count (none)	0	3 (10)
Physician global assessment (0)	0	6 (20)
Patient global assessment (0)	0	5 (17)
Morning stiffness (none)†	1 (3)	11 (39)
Pain assessment (none)	0	5 (17)
HAQ (0)‡	1 (3)	10 (34)
ESR (normal)§	12 (48)	23 (82)
CRP (≤upper limit of normal)§	8 (32)	21 (75)

Values for disease activity (especially laboratory tests) were not available for all patients at all time points.
*Except for laboratory measures, a non-zero score at baseline was required for a patient to be included in this table.
†For morning stiffness, n=29 in placebo group, n=28 in etanercept group.
‡For disability index, n=29 in etanercept group.
§For ESR and CRP tests, n=25 in placebo group, n=28 in etanercept group.

Table 4: **Number (%) of patients with 100% improvement in individual disease-activity measures at 12 weeks**

	Placebo (n=30)	Etanercept (n=30)	p*
Upper respiratory tract events	17 (57%)	17 (57%)	1·0000
Respiratory tract infection	4 (13%)	8 (27%)	0·3334
Pharyngitis	3 (10%)	5 (17%)	0·7065
Rhinitis	4 (13%)	5 (17%)	1·0000
Sinusitis	2 (7%)	3 (10%)	1·0000
Influenza syndrome	6 (20%)	0	0·0237
Injection-site bruise	5 (17%)	6 (20%)	1·0000
Injection-site reaction	1 (3%)	6 (20%)	0·1028
Headache	3 (10%)	4 (13%)	1·0000
Fatigue	0	4 (13%)	0·1124

*Fisher's exact test.

Table 5: **Number of adverse events of all intensities**

Efficacy of etanercept in psoriasis

Etanercept was also effective in improving the skin lesions of psoriasis in the trial (figure 3). Of the 19 patients in each treatment group who were evaluable for psoriasis (≥3% of body surface area involvement), five (26%) of patients in the etanercept group achieved the primary psoriasis endpoint—a 75% improvement in PASI at 12 weeks—compared with no patients in the placebo group (p=0·0154). Similar differences between treatment groups were also seen at the 25% and 50% improvements in the PASI scores; in fact, the median improvement in PASI score was 46·2% in patients receiving etanercept, compared with 8·7% in the placebo group (p=0·0032).

Additionally, the median response of a prospectively defined target lesion in the etanercept group was 50%, compared with none in the placebo group (p=0·0004).

Examination of results in patients who were or were not receiving concomitant methotrexate therapy showed that etanercept was consistently better than placebo in both strata. An imbalance in corticosteroid use and PASI scores existed between the two treatment groups at baseline that might have affected the results of the study. Additional analyses showed that these baseline imbalances did not affect the conclusions of the study (data not shown).

Safety

All 30 of the etanercept patients completed the 12 week course of therapy, and 26 of 30 (87%) placebo patients completed the study. No serious adverse events were reported in the patients receiving etanercept; one serious adverse event occurred in a placebo patient (hospitalised for surgery to correct a rectal tear). No patients developed infections that required hospitalisation or intravenous antibiotics.

The most common adverse events in the study were of the upper respiratory tract and injection-site reactions. No attempt was made to specifically distinguish infectious versus non-infectious adverse events in this study; however, event terms that could potentially refer to infections of the upper respiratory tract are grouped in table 5. Injection-site reactions were mild and well tolerated. No adverse events occurred in a significantly greater proportion in the etanercept group relative to the placebo group.

Discussion

Few double-blind, placebo-controlled trials have been done in patients with psoriatic arthritis. Most therapies used to treat psoriatic arthritis have been attempted because they showed benefit in patients with rheumatoid arthritis or were therapies that yielded apparent benefit

in open uncontrolled trials. The few controlled trials with patients with psoriatic arthritis have yielded inconsistent results.[15-20]

Aside from non-steroidal anti-inflammatory agents, methotrexate has become the most commonly used agent in patients with psoriatic arthritis.[17] Early reports described improvement in joint disorder with aminopterin (an analogue of methotrexate).[28,29] One placebo-controlled trial with high-dose methotrexate (1–3 mg/kg) showed improvement in joint disease activity in psoriatic arthritis;[18] a second trial showed only improvement in the physician's assessment of arthritis activity.[19] These studies also showed some improvement in psoriasis, particularly in the percentage of body surface area involved.

Ciclosporin has been compared with methotrexate in an open trial in psoriatic arthritis.[4] This 23-patient trial showed improvement with each therapy; responses in arthritis measures were greater with methotrexate, and psoriasis scores (PASI) improved more with ciclosporin. The study was not powered to show a difference in response between the two active agents. A randomised double-blind trial of ciclosporin in patients with psoriasis showed significant benefit in psoriasis.[2] However, toxicities were a limiting factor.[2,4,5]

Other disease-modifying agents of rheumatic disease, including sulfasalazine and gold therapy, have been assessed in psoriatic arthritis. Few or no benefits were shown in either psoriatic arthritis or psoriasis.[15-20]

There is a need for a new therapy to treat both psoriatic arthritis and psoriasis. Etanercept has been shown in previous trials to be effective against rheumatoid arthritis with no serious toxic effects. In two randomised controlled trials of etanercept (25 mg subcutaneously twice weekly) in patients with active DMARD-refractory rheumatoid arthritis, 59–71% of etanercept patients achieved the ACR20 response at 6 months, compared with 11–23% of placebo patients ($p<0.001$); 39–40% and 3–5% of patients, respectively, achieved the ACR50 response ($p<0.01$).[25,30] Etanercept was well tolerated and showed no evidence of significant toxicity, with mild injection-site reactions being the only adverse event associated with etanercept administration.

This trial shows that etanercept provides clinically significant benefit to patients with active psoriatic arthritis. Contrary to studies with other antirheumatic agents where at most a small number of variables reached significance, etanercept resulted in significant clinical benefit in the composite measures (PsARC, ACR20, and ACR50) and in each individual factor of disease activity. Additionally, psoriasis improved as measured by the PASI and the target-lesion assessment. Although the study population was powered to demonstrate efficacy and was too small to clearly predict the safety of etanercept in patients with psoriatic arthritis and psoriasis, the safety profile of etanercept in these patients was similar to that previously reported in the rheumatoid arthritis population.[25,30]

Further study in this population would be useful to further establish the safety profile of etanercept in psoriatic arthritis and psoriasis. Whether etanercept would improve articular damage measured radiographically should be examined.

The results of this study indicate that blocking tumour necrosis factor in both psoriatic arthritis and psoriasis may offer a new therapeutic option for patients with both diseases.

Contributors

P J Mease, B S Goffe, J Metz, A VanderStoep, and B Finck contributed to the protocol design. Additionally, P J Mease was principal investigator of the study and did the rheumatology assessments, B S Goffe did the dermatology assessments, and J Metz administered the study. D J Burge reviewed the progress of the study and coordinated the data management and analysis. D J Burge and P J Mease wrote the paper; B S Goffe, J Metz, A VanderStoep, and B Finck critically reviewed the paper.

Acknowledgments

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ABSTRACT SUPPLEMENT

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2022

EFFECT OF TNFα BLOCKADE ON SYNOVIAL HISTOLOGY IN SPONDYLOARTHROPATHY. D Baeten, P Demetter, E Kruithof, F Van den Bosch, N Van Damme, C Cuvelier, H Mielants, E M Veys, F De Keyser Belgium

Objectives: Based on the therapeutic benefit of anti-TNFα treatment in 4 patients with SpA associated with Crohn's disease, 21 therapy-resistant SpA patients were treated with infliximab (5mg/kg) at week 0, 2 and 6 in an open label pilot study. There was a dramatic and highly significant improvement of both axial and peripheral symptoms. In order to substantiate the effect on peripheral arthritis and to investigate the immunological mechanisms of TNFα blockade, synovial histology was evaluated in 8 SpA patients.

Methods: Synovial biopsies were obtained at baseline, week 2 and week 12 and used for histology and immunohistochemistry.

Results: Synovial lining layer thickness tended to decrease, with a significant reduction of CD55+ synoviocytes at week 12 ($p=0.025$). In the sublining layer, vascularity was reduced at week 12 ($p=0.020$) with a decreased endothelial expression of VCAM-1 ($p=0.042$) but not ICAM-1, PECAM-1 and E-selectin. Although at week 2 and week 12 the number of neutrophils ($p=0.038$ and $p=0.041$) and CD68+ macrophages ($p=0.020$ and $p=0.026$) in the sublining layer was decreased, the overall degree of inflammatory infiltration remained unchanged. This could be due to the lymphocyte infiltration as only CD4+ cells (but not CD3+, CD45RO+ and CD8+ cells) tended to decrease, while CD20+ lymphocytes ($p=0.038$) and plasma cells ($p=0.042$) were even increased.

Conclusion: The reduction in lining layer thickness, vascularity and infiltration with neutrophils and macrophages confirm the therapeutic potential of TNF-α blockade in SpA. The adhesion molecule expression, T cell infiltration and, most importantly, B cell infiltration contrast with previous observations in RA and suggest different immunomodulatory mechanisms of anti-TNF-α in SpA.

Disclosure:

2023

ETANERCEPT THERAPY FOR IMMUNE-MEDIATED COCHLEOVESTIBULAR DISORDERS: PRELIMINARY RESULTS IN A PILOT STUDY. Mahboob U Rahman, Dennis S Poe, Hyon K Choi Boston, MA

Objective: Auto-immunity has been implicated as a cause of rapidly progressing sensorineural hearing loss and Meniere's Disease, and thus have been referred to as immune-mediated cochleovestibular disorders (IMCVD). IMCVD remains a management challenge for otologists. Anti-rheumatic agents have been used with variable efficacy and sometimes with serious side effects. In this report, we describe the preliminary result of our experience in patients with rapidly progressing sensorineural hearing loss and Meniere's Disease, who have been treated with etanercept. Methods: Twelve patients with rapidly progressing sensorineural hearing loss were treated with etanercept 25mg subcutaneous injection twice a week. Eight out of 12 patients met the American Academy of Otology-Head and Neck Surgery criteria for diagnosis of Meniere's Disease. All the patients responded to glucocorticoids but did not respond adequately to or developed side effects to conventional anti-rheumatic and immunosuppressive therapies. The main outcome measurement was assessment of hearing change by air conduction pure tone audiograms and/or word discrimination. When present, vertigo, tinnitus, and aural fullness were assessed as well. Results: More than 7 month follow-up was available for all patients (range: 7-16 months). Ten out of 12 (83%) patients had improvement or stabilization of hearing and tinnitus, 7 out of 8 (88%) patients who had vertigo and 8 out of 9 (89%) patients who had aural fullness had resolution or significant improvement of their symptoms. The benefit persisted until the last visit (7 to 16 months after starting etanercept). One patient had initial dramatic improvement but deteriorated after 5 months. The patient's hearing was rescued and stabilized by adding leflunomide to the therapeutic regimen. Similarly, 3 other patients required a second anti-rheumatic agent to maintain stability of their hearing. Two patients had worsening of their overall hearing. One of them developed bilateral iritis raising a diagnostic possibility of Cogan's syndrome. Another patient who had improvement of her symptoms, also had symmetric polyarthralgia with low-titer ANA. There were no significant side-effects from the etanercept therapy. Conclusions: Our limited data suggest that etanercept therapy is safe and may be efficacious in carefully selected patients with rapidly progressing sensorineural hearing loss and Meniere's Disease at least in a short-term basis. These preliminary efficacy and safety results appear encouraging enough to warrant further follow-up and studies for better determination of the potential clinical utility of etanercept for IMCVDs.

Disclosure:

2024

ETANERCEPT IN WEGENER'S GRANULOMATOSIS: A SIX-MONTH OPEN-LABEL TRIAL TO EVALUATE SAFETY. J Stone, M Uhlfelder, D Hellmann, S Crook, N Bedocs, G Hoffman Baltimore, MD and Cleveland OH

OBJECTIVE: To evaluate the safety of etanercept (ET; Enbrel®) in the treatment of Wegener's granulomatosis (WG).

METHODS: This unrandomized trial was designed to evaluate the safety of ET (25 mg sq b.i.w.) in combination with standard therapies for WG. All patients met modified ACR Criteria for WG and had active disease, defined by the Birmingham Vasculitis Activity Score for WG (BVAS/WG), within 1 month of entry. ET was added to the standard therapeutic regimens prescribed according to disease severity, and continued for at least 6 months. Although this trial was not intended to assess the clinical efficacy of ET in WG, we evaluated selected measures of clinical response.

RESULTS: Twenty patients (mean age 46.7 years; range 25-73 years) were enrolled, 11 females and 9 males. At entry, all 20 patients had flares of previously-known disease (mean disease duration 63.6 months; range 14-189 months); 14 (70%) had never achieved complete disease remissions. Sixteen patients (80%) had "limited" disease at entry, and 4 patients (20%) had "severe" disease, defined as constituting an immediate threat to either critical organs or the patient's life. Eighteen of the patients (90%) were receiving glucocorticoids and 18 (90%) another immunosuppressive agent (6 cyclophosphamide). Fourteen of 20 patients (70%) had ET added as the only new therapeutic variable. The most common side-effect of ET was injection site reactions in 8 patients (40%; all mild). Two patients had a combined total of 5 hospitalizations (1 patient had 4), but none were attributable to adverse effects of ET. One patient with severe subglottic stenosis developed pneumococcal tracheobronchitis, and another developed *H. zoster* (localized). One patient is being evaluated for semi-invasive aspergillosis. There were no deaths. The mean BVAS/WG at entry was 3.6 (range: 1-8). Nineteen patients remained on treatment at 6 months, the one exception being a patient who developed retro-orbital disease at 4 months. At 6 months, the mean BVAS/WG score had improved to 0.6 ($P < 0.001$; 95% C.I. 2.1-4.0), and the mean daily prednisone dose was reduced from 19 mg to 7.4 mg ($P = 0.023$; C.I. 1.8-21.3). However, persistently active disease (observed in 15 patients, 75%) was common. One patient developed renal involvement and mesenteric vasculitis while taking ET.

CONCLUSIONS: In this open-label trial, ET used in combination with standard treatments was well-tolerated in patients with WG. Adverse events were few. BVAS/WG scores improved significantly at 6 months, but persistently active WG was common. We have begun a randomized trial to investigate the efficacy of ET in WG.

Disclosure: Dr. Stone has served as a consultant to Immunex Corporation.

2025

VALINE/LEUCINE^{247} POLYMORPHISM OF β2-GLYCOPROTEIN I AFFECTS THE REACTIVITY OF ANTI-β2-GLYCOPROTEIN I ANTIBODIES. Shinsuke Yasuda, Tatsuya Atsumi, Kenji Ichikawa, Eiji Matsuura, Keiko Kaihara, Tatsuji Yasuda, Yoshiharu Amasaki, Takao Koike Sapporo and Okayama, Japan

BACKGROUND; Valine/leucine polymorphism at position 247 of β2-glycoprotein I (β2GPI) is reported to be associated with the presence of anti-β2GPI antibodies and to be a possible risk factor for antiphospholipid syndrome (APS). To clarify the consequence of this genetic variation in patients with APS, correlation between genotypes of APS/SLE patients and positivity of β2GPI-dependent anticardiolipin antibody (aCL) was investigated. In addition, reactivity of anti-β2GPI antibodies was characterized using recombinant valine^{247} and leucine^{247} β2GPI proteins.

METHODS; Eighty-six Japanese APS/SLE patients and 61 matched controls were analyzed for the valine/leucine^{247} polymorphism of β2GPI. The genotype was determined by PCR and restriction fragment length polymorphism analysis using Rsa I. β2GPI-dependent aCL was detected by ELISA.

To compare the reactivity of anti-β2-GPI antibodies to valine^{247} β2GPI and that to leucine ^{247} β2GPI, recombinant valine^{247} β2GPI and leucine^{247} β2GPI were expressed by a baculovirus system. Those variants were prepared on the irradiated plates (anti-β2GPI) or cardiolipin-coated plates (aCL) and the reactivity of a series of anti-β2GPI [immunized anti-human β2GPI monoclonals (Cof19-22), autoimmune anti-β2GPI monoclonals (EY1C8, EY2C9 and TM1G2) and IgGs purified from sera of patients with APS] was investigated.

RESULTS; The frequency of valine^{247} allele in the APS/SLE patients positive for β2GPI dependent aCL was slightly higher than that of APS/SLE patients without β2GPI dependent aCL (31.8% vs.22.7%) or that of healthy controls (19.0%).

No difference was found in the reactivity of the immunized anti-β2GPI (Cof19-22) between the two variants. In contrast, reactivity of the autoimmune monoclonals (EY1C8, EY2C9 and TM1G2) to valine^{247} β2GPI was higher than that to leucine^{247} β2GPI. APS patients' IgGs also showed significantly higher binding to the former compared with the latter ($p=.001$), regardless of the patients' genotypes.

DISCUSSION; Autoimmune anti-β2GPI antibodies had stronger reactivity to valine^{247} β2GPI than to leucine^{247} β2GPI. The cryptic B cell epitopes of APS-associated aCLs (=anti-β2GPI) may be present on domain IV. Since the position 247 is located at the beginning of domain V, substitution of amino acid at this position may alter the interaction between domain IV and V, affecting the reactivity of autoantibodies against β2GPI.

Disclosure:

2026

EPITOPE STUDIES WITH POINT MUTATIONS AND DOMAIN DELETIONS OF β_2-GLYCOPROTEIN-I. Stephen W Reddel, Ying Xia Wang, Yong Hua Sheng, Steven A Krilis Sydney, NSW, Australia

Point mutations of β_2-glycoprotein I (B2GPI) provide a method for more precise determination of the epitope(s) of autoantibodies from patients with APS. Previous studies using B2GPI domain deleted mutants have suggested that epitopes may be found on domains I or IV.

Three recombinant B2GPI proteins with non-conservative single amino-acid mutations in domain I (G40E, R43G, T50A) plus wild type B2GPI and B2GPI mutants with either domain 1 or 5 deleted were used. All proteins were expressed with a his_6-tag. The sera from 8 normal controls; and 18 patients with anti-B2GPI antibodies demonstrated by commercially available ELISA, were screened by ELISA using the wild type, point mutated and domain mutated B2GPI proteins.

2/18 patients showed at least 40% decrease, and 7/18 patients showed at least 20% decrease, in binding to one or more point mutated B2GPI proteins. Rabbit polyclonal anti-B2GPI antibodies and mouse monoclonal anti-domain 5 had no difference in binding, suggesting equivalent antigen coating. Individual serum samples with decrease in binding to the mutations at positions 40 & 43 did not tend to show decreased binding to the muatution at position 50, and vice versa, suggesting that these are non-overlapping epitopes. Only 2/18 patients showed any binding above control to the domain 2-5 mutant, whereas 15/18 patients showed at least 60% binding to the domain 1-4 mutant compared to the wild-type. Mouse monoclonal anti-domain 4 binding was equivalent to both domain mutants.

Previous studies of epitopes for anti-B2GPI antibodies have used domain deleted mutants. The failure to bind when a domain is deleted may be due to the epitope being located within that domain, but it could also be explained by the disruption of normal protein folding elsewhere. We used the minimum possible alteration to a protein: a single point mutation in a single surface exposed amino acid; unlikely to change folding in the other domains. 50% (9/18) of the patient serum samples had diminished binding to one or more mutants. Our study confirms that domain I of B2GPI contains important epitopes for the binding of clinical anti-B2GPI antibodies.

Disclosure:

2027

THROMBUS ENHANCEMENT AND ENDOTHELIAL CELL ACTIVATION *IN VIVO* BY CMV PEPTIDE-INDUCED ANTIPHOSPHOLIPID ANTIBODIES. Azzudin E Gharavi, Silvia S Pierangeli, Ricardo G Espinola, Xiaowei Liu, Eon N Harris Atlanta, GA

Introduction: We induced antiphospholipid (aPL) antibodies in normal mice by immunization with TIF1 a 19 amino acid PL- binding peptide from human cytomegalovirus (CMV) with sequence similarity to the fifth domain of $\beta_2$glycoprotein I (β_2GPI). Monoclonal aPL antibodies were developed from their spleen cells bind cardiolipin in the presence of β_2GPI, have lupus anticoagulant activity and upregulated expression of ICAM-1, VCAM-1 and E-selectin in vitro. Objectives: to evaluate the in vivo effects on thrombus formation and endothelial cell (EC) activation by CMV peptide-induced aPL antibodies. Methods: Two groups of CD-1 male outbred normal mice were injected each with one of the two monoclonal IgM aPL (D3/AC10 or F3/AA4), the third group were injected with control irrelevant murine monoclonal IgM. Mice received 10 μg IgM at 0 hour 48 hours later, and had surgery after 72 hours of the first injection. Mice were anesthetized, the right femoral vein was exposed.The vein was pinched with a standard pressure to introduce an injury and to induce a clot.Clot formation and dissolution in the transilluminated vein were visualized with a microscope equipped with a closed-circuit video system. Activation of endothelial cells was assessed by direct visualization of adhesion of white cells in the microcirculation of the exposed cremaster muscle of mice. The number of leukocytes sticking within five different venules (diameter 25-35 mm). This was defined as those leukocytes that remained stationary for a period of at least 30 seconds.

Results: Mice injected with monoclonal aPL had significantly larger clots that lasted longer. The size of the clots were 2608 ± 1142 and 1820 ± 911 μm^2

for D3/AC10 and F3/AA4, respectively when compared to control group (467.7 ± 183 μm^2) The adhesion of leukocytes to endothelium was also significantly enhanced in mice treated with D3/AC10 or F3/AA4 monoclonal antibodies (14.7 ± 3.0 and 9.5 ± 2.3) compared to control treated mice (4.9±2.1). Conclusion: These findings showed that pathogenic aPL antibodies can be generated by immunization with a viral peptide that has sequence similarity with the phospholipid binding site of β_2GPI (GDKV). This suggest a possible mechanism of induction of autoimmune pathogenic aPL antibodies after incidental exposure to viruses and has important implications in better understanding the origins of APS.

Disclosure:

Meniere's Disease. All the patients responded to glucocorticoids but did not respond adequately to or developed side effects to conventional anti-rheumatic and immunosuppressive therapies. The main outcome measurement was assessment of hearing change by air conduction pure tone audiograms and/or word discrimination. When present, vertigo, tinnitus, and aural fullness were assessed as well. Results: More than 7 month follow-up was available for all patients (range: 7-16 months). Ten out of 12 (83%) patients had improvement or stabilization of hearing and tinnitus, 7 out of 8 (88%) patients who had vertigo and 8 out of 9 (89%) patients who had aural fullness had resolution or significant improvement of their symptoms. The benefit persisted until the last visit (7 to 16 months after starting etanercept). One patient had initial dramatic improvement but deteriorated after 5 months. The patient's hearing was rescued and stabilized by adding leflunomide to the therapeutic regimen. Similarly, 3 other patients required a second anti-rheumatic agent to maintain stability of their hearing. Two patients had worsening of their overall hearing. One of them developed bilateral iritis raising a diagnostic possibility of Cogan's syndrome. Another patient who had improvement of her symptoms, also had symmetric polyarthralgia with low-titer ANA. There were no significant side-effects from the etanercept therapy. Conclusions: Our limited data suggest that etanercept therapy is safe and may be efficacious in carefully selected patients with rapidly progressing sensorineural hearing loss and Meniere's Disease at least in a short-term basis. These preliminary efficacy and safety results appear encouraging enough to warrant further follow-up and studies for better determination of the potential clinical utility of etanercept for IMCVDs.

Disclosure:

X **2024**

ETANERCEPT IN WEGENER'S GRANULOMATOSIS: A SIX-MONTH OPEN-LABEL TRIAL TO EVALUATE SAFETY. J Stone, M Uhlfelder, D Hellmann, S Crook, N Bedocs, G Hoffman Baltimore, MD and Cleveland OH

OBJECTIVE: To evaluate the safety of etanercept (ET; Enbrel®) in the treatment of Wegener's granulomatosis (WG).

METHODS: This unrandomized trial was designed to evaluate the safety of ET (25 mg sq b.i.w.) in combination with standard therapies for WG. All patients met modified ACR Criteria for WG and had active disease, defined by the Birmingham Vasculitis Activity Score for WG (BVAS/WG), within 1 month of entry. ET was added to the standard therapeutic regimens prescribed according to disease severity, and continued for at least 6 months. Although this trial was not intended to assess the clinical efficacy of ET in WG, we evaluated selected measures of clinical response.

RESULTS: Twenty patients (mean age 46.7 years; range 25-73 years) were enrolled, 11 females and 9 males. At entry, all 20 patients had flares of previously-known disease (mean disease duration 63.6 months; range 14-189 months); 14 (70%) had never achieved complete disease remissions. Sixteen patients (80%) had "limited" disease at entry, and 4 patients (20%) had "severe" disease, defined as constituting an immediate threat to either critical organs or the patient's life. Eighteen of the patients (90%) were receiving glucocorticoids and 18 (90%) another immunosuppressive agent (6 cyclophosphamide). Fourteen of 20 patients (70%) had ET added as the only new therapeutic variable. The most common side-effect of ET was injection site reactions in 8 patients (40%; all mild). Two patients had a combined total of 5 hospitalizations (1 patient had 4), but none were attributable to adverse effects of ET. One patient with severe subglottic stenosis developed pneumococcal tracheobronchitis, and another developed *H. zoster* (localized). One patient is being evaluated for semi-invasive aspergillosis. There were no deaths. The mean BVAS/WG at entry was 3.6 (range: 1-8). Nineteen patients remained on treatment at 6 months, the one exception being a patient who developed retro-orbital disease at 4 months. At 6 months, the mean BVAS/WG score had improved to 0.6 ($P < 0.001$; 95% C.I. 2.1-4.0), and the mean daily prednisone dose was reduced from 19 mg to 7.4 mg ($P = 0.023$; C.I. 1.8-21.3). However, persistently active disease (observed in 15 patients, 75%) was common. One patient developed renal involvement and mesenteric vasculitis while taking ET.

CONCLUSIONS: In this open-label trial, ET used in combination with standard treatments was well-tolerated in patients with WG. Adverse events were few. BVAS/WG scores improved significantly at 6 months, but persistently active WG was common. We have begun a randomized trial to investigate the efficacy of ET in WG.

Disclosure: Dr. Stone has served as a consultant to Immunex Corporation.

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ABSTRACT SUPPLEMENT

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SYSTEMATIC AND COHESIVE CLASSIFYING AND RANKING OF DISEASE ASSOCIATED GENE EXPRESSION IN RHEUMATOID ARTHRITIS (RA). Matthias Schneider, Richard Glynne, Ben Ostendorf, Thomas Pauly, Ursula Jeffry, Johannes Friemann, Ghassan Ghandour, Richard Murray Duesseldorf, Germany; South San Francisco, CA and Berlin, Germany

The introduction of TNFa blockade in clinical practice completed the initial step in of a pathophysiologically based approach in RA therapy. Extended basis for pathology related diagnostics and treatment strategies is offered by analysing oligonucleotid-chips, but the productive use of highly parallel gene expression technologies necessitates the ability to manage and evaluate such data in an appropriate biological context of the acquired samples. We describe a systematic approach to evaluate human synovial pathologies (12 RA (7/12 early, untreated) and 8 non-RA samples) by a comprehensive screen of biopsies versus normal human tissues and cells. All tissues and cells were expression profiled using a novel single Affymetrix DNA array displaying 36,000 current gene and EST clusters. Samples were taken by arthrotomy/-arthroscopy indicated by various indications or by mini-arthroscopy of MCPs. Greater than 2 million independent gene measurements were processed and analyzed on a unique bioinformatics system that allows confidence limits to be imposed on an algorithm calculation determining the presence or absence of each gene or EST investigated. All chip data were normalized in the context of the pathological samples as well as data from a normal human "Body Atlas", a reference database of 15 normal human tissues and 3 cell lines where mRNA from each normal sample was independently profiled across the same 36,000 genes and ESTs. Flexible queries were designed such that recognizable gene expression, such as that of MMPs and chemokines, was detected. These data, fulfilling the rules of a query, provided an intrinsic ranking system to evaluate other known genes or ESTs, also fulfilling the rules of the queries. Examples of new associations, such as the transcription factor Kriesler and a series of ESTs are presented. This process flow of information represents a systematic and cohesive means of classifying and ranking disease associated gene expression that provides an annotated disease, normal, and cell type specific context, capable of keeping pace with genome-wide sequence identification efforts.

Disclosure:

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THE HUMAN INTERLEUKIN-10 (IL-10) LOCUS: MAPPING OF NOVEL STRUCTURAL HOMOLOGUES OF IL-10 REVEALS A CLUSTER OF FOUR IL-10 FAMILY MEMBERS WITHIN APPROXIMATELY 140KB ON CHROMOSOME 1Q31-32. J Eskdale, D Kube, J Peat, G Gallagher United Kingdom and Germany

Human IL10 is a cytokine with a broad range of functions, in health and disease. In the autoimmune disease systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA), IL10 contributes to disease susceptibility and severity at both functional and genetic levels. We have previously determined the sequence of 4000bp 5' of the human IL10 ATG and defined several polymorphic elements (both microsatellites and SNPs) and used these to understand the locus, its functional capacity and its disease associations. We have now prepared a preliminary map of the region surrounding IL10.

We firstly identified and cloned a novel homologue of IL10 (termed IL19) and obtained its genomic sequence as well as its 5' and 3' UTR regions. We obtained similar data for a second human IL-10 homologue, MDA7. In addition, we extended our known sequence for IL10 to 9kb 5' of the initial ATG. Next, we identified a previously anonymous cDNA as a third novel IL10 family member and demonstrated its presence in the region. In parallel, we used the PAC clone library from the MRC-HGMP resource centre to identify genomic clones which contained these IL-10 family genes and showed that at least IL10, IL19 and MDA7 existed within a distance of 140kb. Finally, we aligned our new sequence data and the known cDNA and genomic data with unfinished, un-annotated unordered genomic sequence from the human chromosome one sequencig project, in order to order the genes and obtain a preliminary map of this area, which we term "the human IL10 cluster".

The cluster lies at 1q31-32 and radiation hybrid mapping places the IL10 gene very close to the framework marker WI-9640.

IL19, IL10 and MDA7 are all found on a single PAC clone, but IL10 and MDA7 have also been found on a PAC without IL19 while IL10 and IL19 have also been found without MDA7, suggesting an ordering of IL19-IL10-MDA7. The new homologue cDNA overlaps the 9kb 5' of IL10 and so lies between IL19 and IL10. Therefore, although we do not yet have complete sequence of the entire 140kb, we have sufficient information to order the genes thus:

– IL19 -//-IL20 - IL10 -//- MDA7 –

We have examined the expression of all four IL10 family members in human PBMCs and shown that LPS and PHA stimulate the production of mRNA form all four genes, although the timing of induction varies, with MDA7 being later and IL19 being earlier than IL10. Thus, we have shown that a new cluster of IL10 homologues, all capable of induction in PBMCs, lies within 140kb at 1q31-32.

Disclosure:

ACR Abstract Concurrent Session
RA Therapy – New and Novel Therapies

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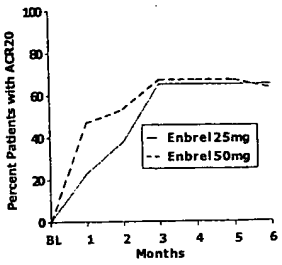
Wednesday, November 1, 2000, 4:00 – 5:30 PM

RANDOMIZED CONTROLLED TRIAL OF 25 MG VS. 50 MG ENBREL® (ETANERCEPT) TWICE WEEKLY IN RHEUMATOID ARTHRITIS (RA). Michael Schiff, Philip Mease, Michael Weinblatt, Larry Moreland, Daniel Burge Denver, CO; Seattle, WA; Boston, MA and Birmingham, AL

Background: Enbrel provides substantial clinical benefit without significant toxicity to RA patients. Previous trials have demonstrated that 25 mg Enbrel twice weekly is significantly more effective than 10 mg twice weekly. This trial explored whether a higher dose of Enbrel is safe and provides additional efficacy for RA patients.

Methods: 77 patients were randomized in a 1:2 ratio at 4 centers to receive 25 mg or 50 mg Enbrel SC twice weekly for 6 months. Efficacy and safety assessments were obtained at baseline, 1, 2, 3, and 6 months.



Results: 23 of 26 (88%) patients on 25 mg Enbrel completed the trial compared to 43 of 51 (84%) in the 50 mg group. In the 25 mg group, no patients discontinued due to adverse events compared with 4 in the 50 mg group. Two of 26 patients (8%) in the 25 mg group and 3 of 51 (6%) in the 50 mg group discontinued due to lack of efficacy. Adverse events were similar in the 2 groups. No patient had a serious infection. Response rates at 6 months were similar in the 2 groups, although the response was more rapid in the higher dose group (see figure). At 6 months, the ACR20, ACR50, and ACR70 were 65%, 38%, and 15% in the 25 mg group compared to 63%, 37%, and 16% in the 50 mg group.

Conclusions: Enbrel was safe and well tolerated when administered to RA patients at doses of 25 mg or 50 mg twice weekly. Although patients in the 50 mg group achieved more rapid results, the two doses were equally effective at 6 months.

Disclosure: This study was supported by a grant from Immunex Corporation.

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A DOSE ESCALATION STUDY DESIGNED TO DEMONSTRATE THE SAFETY, TOLERABILITY AND EFFICACY OF THE FULLY HUMAN ANTI-TNF ANTIBODY, D2E7, GIVEN IN COMBINATION WITH METHOTREXATE (MTX) IN PATIENTS WITH ACTIVE RA. Michael Weisman, Edward Keystone, Harold Paulus, Michael Weinblatt, Daniel Furst, Larry Moreland, Raja Velagapudi, Steven Fischkoff, Elliot Chartash San Diego, CA; Toronto, ON, Canada; Los Angeles, CA; Boston, MA; Seattle, WA; Birmingham, AL and Mt. Olive, NJ

Rationale: The fully human anti-TNF antibody D2E7 has been given successfully as sole therapy in patients with active RA. This study was designed to analyze the safety, tolerability and efficacy of IV administered fully human anti-TNF antibody, D2E7, given with MTX to patients with active RA.

Methods: 60 patients, partial responders to MTX therapy, were randomized into 5 escalating treatment groups. After one month, 59 patients were given unblinded study drug, once every other week. Treatment group change or dropout status was determined by clinical response.

Results: 59 patients were given doses of 0.25-5.0mg/kg. Baseline demographics were (mean): Age 52.9yrs, RA duration 15.7yrs, ESR 38.8mm/hr, CRP 2.3mg/dl, # previous DMARDs 3.2/pt. D2E7 was well tolerated, three patients dropped out because of AEs. The terminal half-life ranges from 353-464 h, and serum clearance ranged from 0.009-0.012 L/h; both were dose independent. The average clearance of MTX slightly increased when co-administered with D2E7. Clinical response after 24 weeks is summarized below.

		0.25mg/kg n=6	0.5mg/kg n=7	1.0mg/kg n=21	3.0mg/kg n=12	5.0mg/kg n=12	Total n=58
ACR20*	24wks	67	71	52	67	75	64
ACR50*	24wks	50	43	29	58	67	47
SJC**	24wks	72	71	56	77	76	68
TJC**	24wks	85	86	61	69	80	73

*Percentage of patients with response **Percentage decline from baseline

Conclusion: The results of this study suggest that the fully human anti-TNF antibody, D2E7 is well tolerated, safe and efficacious when given in combination with MTX in patients with longstanding RA. The response is sustained through at least 24 weeks and the clearance of MTX is only slightly increased.

Disclosure: All work was supported by Knoll Pharmaceutical Company, Mt. Olive, NJ

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ETANERCEPT IN STILL'S DISEASE IN THE ADULT. Michael E Weinblatt, Agnes L Maier, Steven S Overman, Philip J Mease, Patricia A Fraser, Ellen M Gravallese Boston, MA and Seattle, WA

Rationale: Systemic onset juvenile rheumatoid arthritis (Still's Disease), an illness marked by recurrent high fever, skin rash, fatigue and polyarthritis, may be progressive and refractory to conventional therapy. A randomized withdrawal trial of etanercept, the p75 TNF receptor fusion protein, demonstrated efficacy in polyarticular JRA (NEJM 342:763,2000) but it has not been studied in adult Still's disease. High levels of expression of both TNF and lymphotoxin α (LTα) have been noted in synovium of systemic onset JRA patients (A&R 39:1703,1996). Etanercept inhibits the activity of TNF and LTα making it an attractive therapeutic agent to use in Still's Disease.

Methods: This initial pilot study was designed as a six month open trial of etanercept in 12 adult patients who met the criteria for systemic onset juvenile rheumatoid arthritis and had active arthritis. This study was designed primarily to evaluate the effects of etanercept on the arthritis of Still's disease. Patients were initially dosed with etanercept 25 mg twice a week, but the dose could be increased to 25 mg three times a week if no improvement in the arthritis was noted by week 8.

Results: The 12 patient's mean age was 36 yrs.(range 19-62 yrs)(10F:2M)with a disease duration of 10.7 yrs (1-17 yrs) and had received a mean of 3.6 (1-7)prior DMARDs. Five had received prior methotrexate and 5 remained on methotrexate (15-25 mg/week) during the study. Ten were on prednisone at a mean dose of 10 mg/day (5 -20 mg /day). At baseline they had a mean of 24±11 painful joints, 19±10 swollen joints and a mean ESR of 52±38 mm/hr. Ten patients successfully completed the study and 2 withdrew due to disease flare at week 7 and at week 13. Four patients increased their etanercept from 25 biw to 25 tiw without any adverse events. Clinical response as defined by ACR 20% response criteria was observed in 8 patients. Of these 8 responders, 5 met ACR 50% and two met ACR 70% responses. There was a 54% improvement in painful joints, a 63% improvement in swollen joints and a 27% improvement in sed rate. In the 3 patients who had systemic features of Still's (fever and rash) improvement in these features was seen in one patient. Except for the 2 pts who withdrew due to flare (rash, fever and arthritis) no other significant adverse events occurred.

Conclusions: In this initial study of Still's disease in the adult etanercept was well tolerated with improvement in the arthritis. This data suggests that additional trials should be performed to determine the effects of TNF inhibitors on Still's disease.

Disclosure: M. Weinblatt, MD is a consultant to Immunex Corp.P.Mease, MD is a consultant and on the speakers bureau of Immunex Corp. This research was supported by a grant from Immunex Corp.

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SUSTAINED IMPROVEMENT IN RHEUMATOID ARTHRITIS FOLLOWING B LYMPHOCYTE DEPLETION. Jonathan C Edwards, Geraldine Cambridge, Maria J Leandro

Purpose: Work on B lymphocyte survival led to the hypothesis that rheumatoid arthritis (RA) is propagated by a vicious cycle in which autoreactive B lymphocytes (usually IgG rheumatoid factor (RF) committed) perpetuate their existence by generating their own antigen (IgG RF) in self-complexing form and by using surface RF to obtain non-specific T cell help. This hypothesis predicts that total/subtotal B lymphocyte depletion may induce long term remission as long as RF levels fall significantly before B lymphocytes return. Surprisingly, B lymphocyte depletion produces no clinically relevant immunosuppression and has proved safe in lymphoma. An open study of B lymphocyte depletion was undertaken in RA.

Methods: 5 patients with refractory erosive seropositive RA, mean duration 22 yrs, all functional grade III, having failed at least 5 disease modifying drugs (DMARDs), stopped all DMARDs and underwent a 3 week course of B lymphocyte depletion using monoclonal anti-CD20 antibody (rituximab 2.1gm), prednisolone (10 days 60mg then 12 days 30mg p.o.) and cyclophosphamide (750mg IV x 2). Mean follow up is 17 months.

Results: All patients achieved ACR50, and three ACR70 (pts 1,2,3), at 6 months. Patients 1,3,5 maintained/extended improvement at 1 year (ACR70,70,70) and show no sign of relapse. Patients 2 and 4 relapsed at 7 and at 9 months, but have also now achieved ACR70 after repeat B lymphocyte depletion with lower doses of anti-CD20. All DMARD were successfully withdrawn and none has been introduced. B lymphocyte counts remained low for several months after depletion. As predicted, relapses coincided with the return of B lymphocytes, and only in those cases where RF titres remained high. Adverse events were limited to respiratory tract episodes in 2 patients with histories of similar episodes, and mild thrombocytopenia down to 100,000 in 1.

Conclusions: These findings suggest that B lymphocyte depletion may be safe and cost-effective therapy for refractory RA. Statistical analysis indicates that a minimum of 48% (binomial, Documenta Geigy, 55% by direct binomial) of further similar patients treated in the same way can be expected, with 95% confidence, to achieve at least ACR50, and ACR70 if retreatment is included. Retreatment does not appear to pose problems. More recent treatments in 10 further patients have been followed by similar early responses and indicate that cyclophosphamide increases the speed of remission and the response rate but is not essential for major improvement and conversion to seronegativity. A formal controlled trial will follow.

Disclosure: Setting up the work described was assisted by a grant in aid from Hoffman la Roche equivalent to 30% of drug costs (£7,000 sterling). A further contractual arrangement has been set up to advise Hoffman la Roche on further work in return for £4oDiscl

...work was supported by Amgen Pharmaceutical Company, Inc. Canada

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ETANERCEPT IS EFFECTIVE IN THE TREATMENT OF POLYMYOSITIS/DERMATOMYOSITIS WHICH IS REFRACTORY TO CONVENTIONAL THERAPY INCLUDING STEROIDS AND OTHER DISEASE MODIFYING AGENTS. Constantine K Saadeh Amarillo, TX

Polymyositis and dermatomyositis or PM/DM are considered autoimmune conditions which involve predominantly the muscles and other organ systems. PM/DM can lead to significant morbidity and mortality. Corticosteroids are considered the initial agents of choice. Several patients either fail to respond or become either resistant or dependent on steroids. Other disease modifying agents (DMA) were tried and have shown some effectiveness such as cyclosporin A, intravenous gamma globulins or IVIG, methotrexate, or azathioprine. PM/DM has been associated with increased levels of soluble tumor necrosis factor (TNF) receptor in the serum. In this report, etanercept, a soluble TNF receptor agonist, was used in four patients when DMA and steroids in combination failed to control the disease. All four female patients responded very well to etanercept. All patients were weaned off steroids except in one patient who did not wish to be on steroids. There were no side effects from etanercept even in one patient who had a positive ANA and positive Raynaud's phenomenon. The first patient was 29 year old who was dependent on prednisone and did not respond to azathioprine. Etanercept was started in February of 1999 and the CPK improved from a baseline of a thousand to normal. The second patient was a 9 year old girl who was in remission after a long illness with the disease at the age of 6. She was in complete remission after using steroids, IVIG and cyclosporin A. The patient had recurrent disease at the age of 9 and etanercept was given by itself. Her muscle strength improved to normal and the CPK dropped from 1200 to a normal range. The third patient was a 59 year old lady who refused to take steroids and had very high CPK in the range of 50,000. She did not respond to methotrexate and cyclosporin A. She did respond very well to etanercept and her CPK dropped to 3000 with improvement in her muscle strength. Later methotrexate was added and her CPK decreased further to 1181. The last patient was 35 year old who had had severe dermatomyositis with Raynaud's phenomenon and responded partially to IVIG and cyclosporin A. However, the patient could not tolerate cyclosporin and had recurrent upper extremity thrombophlebitis because of the portacath used to administer IVIG. The patient responded dramatically to etanercept and has been for the first time without steroids, IVIG and cyclosporin A. Although etanercept is expensive, it is significantly less expensive than IVIG and in patients who are resistant to conventional therapy etanercept in PM/DM can be useful and effective alternative with excellent tolerance.

Disclosure: Wyeth Ayerst speaker and consultant

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ANTI-TNF-BLOCKADE WITH INFLIXIMAB (REMICADE®) IN POLYMYOSITIS AND DERMATOMYOSITIS. Gerald Hengstman, Frank van den Hoogen, Baziel van Engelen, Pilar Barrera, Mihai Netea, Leo van de Putte Nijmegen, The Netherlands

Introduction: Several studies have indicated a prominent role of TNF in the pathogenesis and sustainment of the inflammatory process in inflammatory myopathies such as polymyositis (PM) and dermatomyositis (DM). Therefore, blocking of TNF could have therapeutic value for these diseases. Herein, we report the preliminary results of two patients with an inflammatory myopathy, treated with a chimeric anti-TNF monoclonal antibody (Remicade®).Patients and Methods: Patient A is a 51 year old female with a 3 months history of proximal muscle weakness and periorbital erythematous skin lesions. Patient B is a 39 year old female with a 2 months history of proximal muscle weakness. Further examination revealed elevated CK, and characteristic findings on EMG and in muscle biopsy in both patients. Patient A was diagnosed as definite DM, patient B as definite PM, according to the diagnostic criteria of Peter and Bohan. Patients were treated with Remicade®, at a dose of 10 mg/kg body weight, every two weeks. Muscle strength (using fixed myometry to measure maximum voluntary isometric contraction) and EMG were performed before every dose anti-TNF. Muscle biopsy was repeated after the second dose.

Results: Muscle strength improved already after the first injection and reached normal values when measured after the third injection in both patients. In the same time-span, serum CK concentrations decreased from 7533 to 3204 IE/ml in patient A and from 3905 to 1755 IE/ml in patient B. Changes on EMG paralleled clinical improvement and showed less spontaneous activity and larger compound motor action potentials of longer duration. Histological examination of the muscle biopsy taken after the second dose anti-TNF showed a disappearance of necrotic muscle fibers and a decrease of the lymphocytic infiltrates. The skin lesions of patient A vanished after the first administration of anti-TNF. No side effects were observed.

Conclusion: The improvement in disease activity parameters and the lack of side effects observed in these two patients suggests that TNF neutralization might be an effective and safe treatment for inflammatory myopathies. Our results underscore the role of TNF in the pathogenesis of these diseases.

Disclosure: Remicade was kindly provided by Schering-Plough SA/NV Benelux.

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POLYMYOSITIS AND TOXOPLASMOSIS: AN OBSERVATION IN PATIENTS WITH CRUSH SYNDROME. Ayhan Dinc, Salih Pay, Ismail Simsek, Hakan Erdem, Mehmet Tanyuksel, Kayser Caglar, Mehmet Refik Mas Ankara, Turkey

Background: Several studies including a number of case reports have suggested that infection with the *Toxoplasma gondii* is associated with polymyositis (PM). We had evaluated a 32-year-old male PM patient with an extensive muscle involvement leading to proteinuria. Serological investigation showed markedly elevated IgG and IgM titers to *Toxoplasma gondii*. Sabin- Feldman dye test was also positive at a dilution of 1:1024. However, studies of the muscle biopsy specimens and inoculation of Swiss-albino white mice peritoneum with his bone marrow aspirate failed to demonstrate the presence of Toxoplasma trophozoids. He was treated with steroids and methotrexate and followed up for more than two years without any symptom. One possible explanation of this case is of an anamnestic response to antigenic components of Toxoplasma previously sequestered in the muscles, released as result of extensive muscle damage seen in the course of PM. To test this hypothesis we examined serologic responses of 14 earthquake victims with severe crush injury, a cause of non-inflammatory muscle necrosis.

Patients and Methods: 14 patients with severe and extensive crush injury, all of whom had marked elevations in myogenic enzymes and myoglobinuria, and hospitalised for renal insufficiency, were tested for IgM and IgG anti-Toxoplasma antibodies using ELISA at the first and fourth weeks following the injury. 15 healthy volunteers served as controls.

Results: Of the 14 patients with crush injury, and of the 15 healthy subjects, all had negative IgM findings both at the baseline and during the follow-up. Of the 9 crush patients with positive IgG titers at baseline (possibly indicating previous infection), 8 showed a marked increase (At least four-fold) in IgG titers at the 4th week. Six of the healthy subjects had positive IgG values at baseline but none had showed any increase in IgG titers at the test of 4 week later. All patients who were initially negative for IgG remained negative during the follow-up.

Discussion: This observation further supports the suggestion that the antigenic structures of *Toxoplasma gondii* disseminated throughout the skeletal muscles in the course of a previous acute infection may release and lead to an anamnestic response in those PM patients with an extensive muscle damage.

Disclosure:

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CUTANEOUS MANIFESTATIONS AND BREAST CALCINOSIS IN ADULT ONSET DERMATOMYOSITIS (AODM). Eric R Tamesis, Gerald F Palasca, Bernardo Pons-Estel, Mark T DiMarchangelo, Antonio J Reginato Camden, NJ

Introduction: Calcinosis is an infrequent finding in adult onset dermatomyositis

Objective: To describe the cutaneous involvement, and extent of calcinosis in a cohort of patients with Adult Onset Dermatomyositis (AODM).

Patients and Methods: Retrospective chart review of 17 cases of dermatomyositis actively followed by the rheumatology service in a university referral center. All patients met the criteria set by Bohan and Peter for the diagnosis of dermatomyositis which included: progressive proximal and symmetrical muscle weakness, increased concentration of CPK and aldolase, an abnormal electromyogram, an inflammatory muscle biopsy, and characteristic cutaneous disease, were included in the ARTHROS 6.0 database system.

Results: Out of 17 patients with dermatomyositis, 5 patients or 29 % exhibited extensive calcinosis in different distinct patterns. All five patients were women, the patterns seen included calcinosis cutis, subcuticular and deep muscular involvement. The calcifications involved the abdominal wall(3), the extremities(5), the head and neck(1), and even the involvement of the breasts(5). Fourteen out of the seventeen patients had multiple skin manifestations with other cutaneous findings. All required multiple medication combinations including prednisone, azathioprine, methotrexate, hydroxychloroquine, cyclosporine and IVIG in an attempt to control the disease with improvement seen in 13 patients. Calcium channel blockers were also used in attempt to treat the extensive calcification involvement but were unsuccessful. Some patients with the most extensive cutaneous involvement continued to manifest disease activity despite above medications.

Conclusion: Extensive calcification is more common in AODM and is resistant to available therapy than previously thought. Control of disease activity required more medication combinations when there were more cutaneous manifestations present. Breast calcifications only once previously reported was seen in 5 of our patients. These calcifications can show bizarre sheetlike branching or ringlike macrocalcifications. These calcifications would present a problem in attempts at mammographic screening for breast cancer

Disclosure: Speaker Bureau of PANLAR, Pfizer, Merck

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HIGH RESOLUTION LUNG CT IS USEFUL TO PREDICT THE OUTCOME OF INTERSTITIAL PNEUMONIA IN PATIENTS WITH ANTI-JO-1 NEGATIVE POLYMYOSITIS (PM) / DERMATOMYOSITIS (DM). Masaomi Yamasaki, Hidehiro Yamada, Yoshioki Yamasaki, Hiroshi Niimi, Yasuyuki Kurihara, Nobuaki Hama, Yoichi Ichikawa Kawasaki, Kanagawa, Japan

Objective. We have found that anti-Jo-1 negative IP patients with PM/DM have a significantly higher mortality rate than anti-Jo-1 positive patients and that their clinical course is varied. The present study aimed to investigate whether HRCT can predict the outcome of IP complicated in anti-Jo-1 negative PM/DM.

Methods. Twenty-three patients with anti-Jo-1 negative PM/DM who admitted because of progressive IP since 1994 were retrospectively analyzed. HRCT and pulmonary function test were performed before treatment. Clinical and laboratory parameters at the initial presentation were also collected for analysis. Clinical status and cause of death were determined at the end of the observation period. HRCTs were reviewed blindly by two radiologists (H.N, Y.K).

Results. The 23 patients (3 male, 20 female; aged 43-80) were treated with either high-dose steroid or concomitant immunosuppressive drugs (8 cyclosporine, 6 cyclophosphamide). Ten out of 23 patients died of progressive IP. The other patients were alive with a mean follow-up of 38.1 months (5-104). Significantly high mortality rate was found in patients whose dominant HRCT was air-space consolidation plus ground-glass opacity at the initial presentation compared to those with other HRCT findings (70% vs. 23%, $p<0.05$). No other clinical and laboratory parameters discriminated a significant difference between patients with fatal IP and those who survived. Logistic regression analysis indicated that air-space consolidation plus ground-glass opacity on HRCT was a sole independent contributory factor for the fatal IP (risk ratio 19.2 (95% CI; 1.02-342)). This was consistent even after adjusting for treatment.

Conclusion. This study suggests that HRCT findings at the initial presentation predict the outcome of IP in patients with anti-Jo-1 negative PM/DM.

Disclosure: Funded by Dr.Kuniomi Yamasaki

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CHARACTERIZATION OF INTERSTITIAL PNEUMONIA IN ANTI JO-1 ANTIBODY POSITIVE PATIENTS WITH POLYMYOSITIS / DERMATOMYOSITIS. Masaomi Yamasaki, Yoshioki Yamasaki, Hidehiro Yamada, Hiroshi Niimi, Yasuyuki Kurihara, Nobuaki Hama, Yoichi Ichikawa Kawasaki, Japan

Objective. To investigate clinical characteristics of interstitial pneumonia (IP) in anti-Jo-1 antibody positive patients with polymyositis/dermatomyositis (PM/DM) .

Methods. Thirty-three patients with PM/DM suffering from progressive IP were treated at our hospital and followed up at least one year since 1992. All these patients were subjected to detail general and respiratory assessment and high resolution CT (HRCT). Serial HRCTs were evaluated independently by two radiologists(H.N,Y.K) in a blinded manner. The subjects consisted of ten (6PM/4DM) patients with anti-Jo-1 antibody and they were compared with 23 (1PM/22DM) anti-Jo-1 negative patients.

Results. Mean follow-up period was 26.8 months (Jo-1 (+) group; 39.3 months and Jo-1 (-); 21.2 months). The mortality rate was significantly lower in Jo-1 (+) group than Jo-1 (-) ($p<0.02$). No significant differences were found in respiratory symptoms and signs at the initial presentation between the two groups. The dominant findings of the initial lung HRCT were ground glass opacity and/or air-space consolidation in 90% of Jo-1 (+) patients, while HRCT findings of Jo-1 (-) were quite heterogeneous. All the Jo-1 (+) patients initially showed an improvement of IP with steroid therapy alone, but a higher recurrence rate was evident in Jo-1 (+) group (9 of 10) than negative group (2 of 23). The dominant HRCT findings at the recurrence were linear opacity and/or ground glass opacity. The recurrent IP was treated with steroid alone in four patients and cyclosporine in another five. Both of them did not show a significant improvement and slowly progressed.

Conclusion. This study suggests that IP in Jo-1 (+) patients have a unique clinical course of IP. Relapse of slowly progressive IP is frequent and these cases may require immunosuppressive agents in the early course of the disease.

Disclosure: funded by Dr. Kuniomi Yamasaki

[redacted] because of the po[redacted]ath used to administer IVIG. The patient responded dramatically to etanercept and has been for the first time without steroids, IVIG and cyclosporin A. Although etanercept is expensive, it is significantly less expensive than IVIG and in patients who are resistant to conventional therapy etanercept in PM/DM can be useful and effective alternative with excellent tolerance.

Disclosure: Wyeth Ayerst speaker and conultant

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ANTI-TNF-BLOCKADE WITH INFLIXIMAB (REMICADE®) IN POLYMYOSITIS AND DERMATOMYOSITIS. Gerald Hengstman, Frank van den Hoogen, Baziel van Engelen, Pilar Barrera, Mihai Netea, Leo van de Putte Nijmegen, The Netherlands

Introduction: Several studies have indicated a prominent role of TNF in the pathogenesis and sustainment of the inflammatory process in inflammatory myopathies such as polymyositis (PM) and dermatomyositis (DM). Therefore, blocking of TNF could have therapeutic value for these diseases. Herein, we report the preliminary results of two patients with an inflammatory myopathy, treated with a chimeric anti-TNF monoclonal antibody (Remicade®).Patients and Methods: Patient A is a 51 year old female with a 3 months history of proximal muscle weakness and periorbital erythematous skin lesions. Patient B is a 39 year old female with a 2 months history of proximal muscle weakness. Further examination revealed elevated CK, and characteristic findings on EMG and in muscle biopsy in both patients. Patient A was diagnosed as definite DM, patient B as definite PM, according to the diagnostic criteria of Peter and Bohan. Patients were treated with Remicade®, at a dose of 10 mg/kg body weight, every two weeks. Muscle strength (using fixed myometry to measure maximum voluntary isometric contraction) and EMG were performed before every dose anti-TNF. Muscle biopsy was repeated after the second dose.

Results: Muscle strength improved already after the first injection and reached normal values when measured after the third injection in both patients. In the same time-span, serum CK concentrations decreased from 7533 to 3204 IE/ml in patient A and from 3905 to 1755 IE/ml in patient B. Changes on EMG paralleled clinical improvement and showed less spontaneous activity and larger compound motor action potentials of longer duration. Histological examination of the muscle biopsy taken after the second dose anti-TNF showed a disappearance of necrotic muscle fibers and a decrease of the lymphocytic infiltrates. The skin lesions of patient A vanished after the first administration of anti-TNF. No side effects were observed.

Conclusion: The improvement in disease activity parameters and the lack of side effects observed in these two patients suggests that TNF neutralization might be an effective and safe treatment for inflammatory myopathies. Our results underscore the role of TNF in the pathogenesis of these diseases.

Disclosure: Remicade was kindly provided by Schering-Plough SA/NV Benelux.

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POLYMYOSITIS AND TOXOPLASMOSIS: AN OBSERVATION IN PATIENTS WITH CRUSH SYNDROME. Ayhan Dinc, Salih Pay, Ismail Simsek, Hakan Erdem, Mehmet Tanyuksel, Kayser Caglar, Mehmet Refik Mas Ankara, Turkey

Background: Several studies including a number of case reports have suggested that infection with the *Toxoplasma gondii* is associated with polymyositis (PM). We had evaluated a 32-year-old male PM patient with an extensive muscle involvement leading to proteinuria. Serological investigation showed markedly elevated IgG and IgM titers to *Toxoplasma gondii*. Sabin- Feldman dye test was also positive at a dilution of 1:1024. However, studies of the muscle biopsy specimens and inoculation of Swiss-albino white mice peritoneum with his bone marrow aspirate failed to demonstrate the presence of Toxoplasma trophozoids. He was treated with steroids and [redacted]

Arthritis & Rheumatism

ABSTRACT SUPPLEMENT

2000 ANNUAL SCIENTIFIC MEETING

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Volume 43, No. 9 (Supplement) September 2000

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ALLELE- AND ANTIGEN-SPECIFIC TREATMENT OF RHEUMATOID ARTHRITIS: FINAL RESULTS FROM A DOUBLE BLIND PLACEBO CONTROLLED PHASE 1 TRIAL. Alan J Kivitz, Harold E Paulus, Nancy J Olsen, Michael H Weisman, Eric L Matteson, Daniel E Furst, Ronald Van Vollenhoven, Mark C Genovese, James D Anderson, Stanley B Cohen, Nathan Wei, Jan H Meijerink, Cindy A Jacobs, Simonetta Mocci, Jan E Baughman, Arthur F Kavanaugh Ducansville, PA; Los Angeles, CA; Nashville, TN; Rochester, MN; Seattle, WA; Stockholm, Sweden; Stanford, CA; Wichita, KS; Dallas, TX; Frederick, MD; Oss, The Netherlands and La Jolla, CA

Purpose: To evaluate the safety, pharmacokinetics and preliminary clinical activity of AG4263 in RA patients (pts) with active disease despite concomitant methotrexate (MTX). AG4263 is a soluble complex of native HLA-DR4 and a synthetic 13-mer peptide (CDP263) derived from human cartilage glycoprotein 39 (HC gp-39). HC gp-39 is a putative immunodominant RA autoantigen.

Methods: 31 HLA-DR4 (β*0401) positive RA pts who had failed ± 1 DMARD and had active disease despite stable concurrent MTX (5-20 mg/week) were randomized to receive 7 IV doses of AG4263 (n=24) or placebo (P; n=7) over 6 weeks. AG4263 was given in a dose escalating schedule (0.5 to 150 μg/kg). *Safety analyses included adverse events (AEs), measures of CD4+/CD8+ counts, T cell reactivity (Tcr) to tetanus toxoid (TT)/PPD, and serum antibody (ab) to HLA-DR4. Clinical activity was assessed using Paulus20 criteria and biological activity was assessed by Tcr to CDP263 using ELISPOT assay. Pharmacokinetics (PK) was assessed at the 150 μg/kg dose.*

Results: 31 pts were enrolled; 3 homozygous and 28 heterozygous for β*0401. 3/7 P and 14/18 treated pts at 60 and 150 μg/kg had baseline Tcr to CDP263. The most common AE was injection site reaction. One grade 3 toxicity (pleuritis) occurred; all others were Grade 1 and 2. There was no loss of Tcr to TT/PPD and no pt developed ab to DR4. CD4+/CD8+ counts remained stable. The mean half-life was 12.5 hours. A total of 16/18 treated pts achieved Paulus20 at any time point, vs. 4/7 P. Response was greatest among CDP263 pts and at the highest doses, with 85.7% of the 150 μg, 28.6% of the 60 μg and 0 % of P pts achieving Paulus20 at Day 28 ($p = .028$).

Conclusions: AG4263 was well tolerated and there was no evidence of generalized immune suppression. There was a greater trend toward loss of Tcr to CDP263 among treated pts at 60 and 150 μg/kg vs. P pts and a possible correlation with shift to negative reactivity to CDP263 and achievement of response. Further studies of additional treatment paradigms are warranted.

Disclosure: Research funded by NV Organon and Corbus Corporation.

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EFFICACY AND SAFETY OF FK506 IN RHEUMATOID ARTHRITIS(RA): A 16-WEEK, DOUBLE-BLIND, RANDOMIZED STUDY. H Kondo, S Irimajiri, S Sugawara, H Hashimoto, S Uchida, M Hara, T Abe, For the Japanese FK506 RA Study Group Sagamihara, Kanagawa, Japan

Objective: To evaluate the efficacy and safety of FK506(tacrolimus) in active RA patients who are resistant to DMARDs therapy and to find the optimal dose.

Methods: 212 patients with DMARDs-resistant RA were enrolled in this doubleblind, multicenter, randomized, placebo-controlled study and allocated to 3 groups (1.5mg/day n=68, 3mg/day n=70, placebo n=74). They were treated for 16 weeks and allowed to continue taking prednisolone≤6as than 5mg/day) and/or one kind of NSAIDs during the trial. Clinical assessment was based on the ACR20 response criteria.

Results: A statistically significant difference in ACR20 response rate was seen between the 3mg group and the placebo group, but not between the 1.5mg group and the placebo group. Most of measures of ACR20 criteria were improved significantly in the 1.5mg and 3mg groups as compare to the placebo group.

	1.5mg	3mg	Placebo
ACR20 response(%)	24.6	48.3**	14.1
Δ Tender Joint C.	-3.3 ± 6.2*	-5.3 ± 6.6**	-0.4 ± 7.2
Δ Swollen Joint C.	-3.6 ± 4.5*	-4.2 ± 5.5**	-1.3 ± 4.6
Δ Patient[a]	-9.8 ± 25.8**	-20.4 ± 24.6***	3.1 ± 26.0
Δ Physician[a]	-15.8 ± 21.5**	-29.6 ± 22.4***	-3.7 ± 26.4
Δ CRP(mg/dl)	-0.36 ± 2.05	-1.77±2.40***	0.36 ± 3.37
Δ ESR(mm/h)	-0.9 ± 19.3**	-14.7 ± 19.0***	7.1 ± 24.0

*P<0.05, **P<0.01, ***P<0.001 vs placebo, [a]global assessment of disease activity

Adverse events were observed in 46.3%, 61.3% and 64.4% of the patients in the placebo, 1.5mg and 3mg groups, respectively(no significant difference). The main adverse drug reactions were increases in values indicative of renal function and gastrointestinal symptoms, but most of these were mild and tolerable.

Conclusions: Our findings demonstrate a favorable FK506 dose response in patients with DMARDs-resistant RA. The optimal daily dose may be 3mg.

Disclosure: This study was supported by Fujisawa Pharmaceutical Co.,LTD., Osaka, Japan

ACR Abstract Concurrent Session
Treatment Issues in Scleroderma

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Wednesday, November 1, 2000, 4:00 - 5:30 PM

HIGH-DOSE IMMUNOSUPPRESSIVE THERAPY (HDIT) WITH AUTOLOGOUS STEM CELL TRANSPLANTATION (SCT) FOR SYSTEMIC SCLEROSIS (SSc): RESULTS IN THE FIRST 8 PATIENTS. D E Furst, P McSweeney, R Nash, L Holmberg, F Viganego, L Nelson, M Wener, K McDonagh, L Crofford, J Storek, G Agee, K Ryan, K Prather, M Mayes, R Dansey, K Sullivan

Objectives: To investigate the safety and potential efficacy of HDIT and SCT for severe SSc in the initial 8 patients treated within the National Collaborative Study for SCT in Autoimmune Diseases.

Methods: Autologous peripheral blood stem cells (PBSC) were CD34-positive selected after mobilization with G-CSF (16μg/Kg /day). HDIT consisted of 800 cGy total body irradiation, cyclophosphamide 120 mg/kg, horse-derived ATG 90mg/kg, and re-infusion of PBSC.

Patients: 8 (7 female, 1 male) poor prognosis patients affected by early (≤3 years) diffuse (mRodnan skin score >16) SSc, with predefined internal organ involvement or rapidly progressive disease were included. The median age was 41 years (23-61) and the median disease duration was 21 months (6-40).

Efficacy: 6 patients were evaluable for response with a median follow-up of 711 days (431-1040). At one year post-transplant, the mean skin score improved from 38.2±10(SD) to 21.2±6 (p=0.002); *mean mHAQ improved from 1.85±0.9 to 0.24±0.3 (p=0.005),* whereas FVC and DLCO remained stable (mean % FVC: 73.5±6 to 74.2±6, ns; mean % DLCO: 56.5±11.5 to 50.1±13.5, ns).

Adverse Effects : 2 patients died at days 58 and 79 from interstitial pneumonitis possibly related to radiation toxicity and underlying lung disease. Infections included H simplex flares (n=2), UTI (n=1) and central line infection (n=1). Other AEs included severe mucositis (n=1), renal crisis (n=1), reactions to ATG infusion (n=1), paranoid reaction to Ativan (n=1), thrombophlebitis (n=1), depression (n=2) and FUO (n=1).

Conclusion: Among poor prognosis SSc patients who survived at least 1 year after SCT, skin scores and mHAQs improved significantly, while pulmonary function was stable. Severe pre-existing pulmonary disease may predispose to pulmonary toxicity after HDIT. In the next 10 enrolled patients, lung shielding to 200 cGy irradiation was employed and no further episodes of interstitial pneumonitis have occurred. The safety and efficacy of HDIT with lung shielding is currently being evaluated.

Disclosure:

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ASSESSMENT OF CONSTRUCT VALIDITY OF THE EUROPEAN SCLERODERMA STUDY GROUP (ESsSG) ACTIVITY CRITERIA FOR SYSTEMIC SCLEROSIS. Gabriele Valentini, Alessandra Della Rossa, Salvatore D'Angelo, Walter Bencivelli, Alan J Silman, Carol M Black, Laszlo Czirjak, Hendik Nielsen, Frank HJ Van den Hoogen, Panayiotis J Vlachoyiannopoulos, Stefano Bombardieri, for ESsSG

Objective To assess the construct validity of 3 recently developed sets of weighted activity criteria for systemic sclerosis (SSc): the first set for SSc as whole (TSS > 20 = 1; scleredema = 1; Δ-skin = 2; digital necrosis =1; interstitial lung disease = 1; Δ-heart-lung = 2; ESR > 30 =1; hypergammaglobulinemia =1); the second set for diffuse SSc (dSSc) (Δ-skin = 3; interstitial lung disease = 2; pulmonary hypertension = 1; Δ-heart-lung =3; ESR > 30 = 1); and the third set for limited SSc (lSSc) (scleredema = 1; Δ-skin = 2; digital necrosis =1; Δ-vasc = 1; pulmonary hypertension = 2; Δ-heart-lung =1; ESR > 30 =1; hypocomplementemia = 1) (maximal value for each set = 10) (Valentini et al., Arthritis Rheum 1998; 41(S9), 1105; Valentini et al. Ann Rheum Dis 1999; Suppl, 185).

Methods Out of 318 clinical charts available for the study, 30 with different activity index values (from 0 to 8) were sent to 5 ESsSG experts who were asked to rank the clinical charts from 1 to 30 according to their blind evaluation of disease activity. The correlations among the activity indexes calculated for the 30 patients and the ranks assigned by the 5experts were investigated by Spearman's test.

Results The calculated index for the whole series was significantly correlated with the rank assigned by 4 out of the 5 experts (rho from 0.574 to 0.649; $p < 0.01$). The calculated index for dSSc was significantly correlated with the rank assigned by 4 of the 5 experts (rho from 0.488 to 0.848; $p < 0.05$ or $p < 0.01$). The calculated index for lSSc was significantly correlated with 3 of the 5 assigned ranks (rho from 0.670 to 0.882; $p < 0.05$ or $p < 0.01$).

Conclusion The construct validity of 3 recently developed sets of activity criteria for SSc as whole, for lSSc and for dSSc is supported by the results of this blind evaluation of disease activity by 5 external experts using the data contained in the clinical charts of 30 real patients. The criteria, however, await validation based on a new series of patients.

Disclosure:

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ETANERCEPT AS TREATMENT FOR DIFFUSE SCLERODERMA: A PILOT STUDY. Michael H Ellman, Patricia A MacDonald, F Ann Hayes Chicago, IL and Seattle, Washington

Ten patients with diffuse scleroderma (DS) of less than 5 years duration were treated with etanercept 25 mg administered subcutaneously twice weekly for 6 months (IND# 8303). The Rodnan skin score was the primary outcome measurement; secondary measurements were pulmonary function tests, physician and patient global assesment, oral aperture opening and hand extension and the Modified Health Assessment Questionnaire (HAQ). The patients were allowed to continue current treatments for DS; the maximum prednisone dose allowed was 10 mg/day. Exclusions to entry included a DLCO <45%, hemoglobin below 9.5 Gm/dl, creatinine >1.8 mg/dl and hypertension or other serious illnesses. RESULTS: The patients (9F, 1M) had a mean age of 52.6 years (range 27-71 years) and had DS for a mean of 17 months (range 4 to 58 months). 2 patients were being treated with penicillamine, 2 with minocycline and 4 with prednisone. 4 patients entered the study with digital ulcers. 1 patient with rapidly progressive DS experienced worsening fingertip ulcerations leading to gangrene and discontinued the study after receiving 4 weeks of etanercept. Of the 9 patients who completed the study, 4 patients had Rodnan skin scores that improved approximately 44% (range 27-74%) and 5 patients had skin scores that were unchanged. The digital ulcers improved in the 3 patients with ulcers that remained in the study. The patients that improved had a mean duration of DS 9 months less than those who remained stable. Pulmonary function tests remained stable from month 0 to month 6 (DLCO: 66 to 65%; TLC: 86 to 87%; FRC: 99 to 95%). Patient and physician assessment (0 the worse and 5 best) improved from 3.1 to 4.1 and 2.8 to 4 respectively. The HAQ scores improved from 1.8 to 1.57. Oral aperture and hand extension were unchanged. There were no infections. Etanercept proved safe and easy to self administer; there were no adverse effects other than minor injection site reactions and 4/9 patients had marked skin score improvement; no patient had significant worsening of the Rodnan skin score. CONCLUSION: A double blind study in patients with very early DS, comparing etanercept with placebo is warranted.

Disclosure: Etanercept medication and financial support for data management was provided by Immunex Corporation. F. Ann Hayes, M.D. is a Senior Vice President, Medical Development, Immunex Corp.

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LUNG TRANSPLANTATION IN SCLERODERMA: THE JOHNS HOPKINS EXPERIENCE. Lionel Schachna, Fredrick M Wigley, Barbara White, Allan C Gelber, I Rosas, Jonathan B Orens Bethesda, MD

In the past scleroderma (SSc) was considered a contraindication to lung transplantation. There is little information about the evaluation or outcomes of transplantation in SSc patients.

Of 25 patients with SSc referred for lung transplantation since 1996,only 4 were deemed inappropriate candidates due to comorbid conditions (renal failure[2], severe coronary artery disease[1], implantable defibrillator[1]). 3 patients declined transplantation and routine evaluation is ongoing in 4 patients. Of 7 patients placed on the active waiting list, 1 died and 1 improved with medical management. The table demonstrates the outcomes of 7 consecutive patients with SSc who underwent lung transplantation.

Patient	SSc type	ILD/PHT	Transplant	Outcome/ FVC	Follow-up duration
53WM	lSSc	+/++	SLT	acute rejection	died day 29
37BF	lSSc	+++/-	SLT	alive/53%	15 months
35WF	lSSc	+++/-	SLT	alive/ 71%	15 months
53WF	lSSc	++/+++	SLT	alive/ 67%	15 months
51WM	lSSc	+++/++	HLT	subdural hematoma	died day 57
50WF	lSSc	√+++	SLT	alive/ 110%	3 months
36BF	dSSc	+++/+	BLT	alive/ 40%	2 months

lSSc:limited,dSSc:diffuse; ILD:interstitial lung disease (+ 70-80%,++ 51-69%,+++ ≤50% FVC); PHT: pulmonary hypertension (+ 36-45, ++ 46-55, +++ ≥56mmHg RVSP); SLT/BLT/HLT:single/bilateral/heart lung transplant

There was 1 death directly related to transplantation and 1 died from head trauma after a fall. Of note, 3 long-term survivors have maintained satisfactory FVC (53-71%). Overall 5/7 (71%) are alive (follow-up 2-15 mo).We conclude that most SSc patients who completed an evaluation met criteria for transplantation. The short-term outcome at our center compares favorably with one-year 70% survival reported for lung transplantation overall.

Disclosure:

the 5 assigned ranks [illegible] from 0.670 to 0.882; $p < 0.05$ or [illegible] < 0.01).

Conclusion The construct validity of 3 recently developed sets of activity criteria for SSc as whole, for lSSc and for dSSc is supported by the results of this blind evaluation of disease activity by 5 external experts using the data contained in the clinical charts of 30 real patients. The criteria, however, await validation based on a new series of patients.

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1955

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Ann Rheum Dis 1999;58:(Suppl I) I121–I128

The role of TNFα and lymphotoxin in demyelinating disease

Christopher Lock, Jorge Oksenberg, Lawrence Steinman

Multiple sclerosis (MS) is an inflammatory demyelinating disease of central nervous system (CNS) white matter.[1] The aetiology is unknown but the condition is probably the result of a misdirected immune response against myelin antigens. Pathologically there are multiple plaques or areas of white matter inflammation, demyelination, and glial scarring or sclerosis. In addition to myelin damage, axon loss may occur as there is a close relation between myelin and axon.[2] The inflammatory lesions are disseminated in time and space, and clinically the illness is characterised by relapsing episodes of neurological dysfunction.

In a commonly proposed sequence of events, autoreactive myelin specific CD4+ Th1 are activated in the periphery, probably by non-self antigens with a resemblance to myelin proteins.[3] T cells interact with adhesion molecules, such as selectins and integrins, on the capillary endothelium and then migrate into the brain parenchyma in response to chemotactic signals. Matrix metalloproteases (MMPs) are important in facilitating T cell penetration through the endothelial basement membrane. Microglia and astrocytes reactivate T cells locally in the CNS by presentation of myelin proteins bound to class II MHC molecules. T cells stimulate macrophage activity by release of proinflammatory cytokines such as IL2, IFNγ, tumour necrosis factor α (TNFα) and lymphotoxin (LT). Activated macrophages phagocytose myelin, and damage myelin by release of proteases, nitric oxide metabolites, reactive oxygen species, eicosanoids, complement components and TNFα. Autoantibodies directed against myelin basic protein (MBP) and myelin oligodendrocyte glycoprotein (MOG) are involved in myelin damage.[4 5]

Experimental allergic (or autoimmune) encephalomyelitis (EAE) can be induced in a variety of animal species, including non-human primates, by immunisation with spinal cord homogenates, myelin proteins or their peptide derivatives. A number of different myelin proteins can induce EAE, including proteolipid protein (PLP), MBP, MOG, myelin associated glycoprotein (MAG), and 2'3' cyclic nucleotide 3'-phosphdiesterase (CNP). Immunisation induces brain inflammation accompanied by varied signs of neurological disease. EAE can be adaptively transferred by myelin sensitised T cells. EAE and MS share common clinical, histological, immunological and genetic features, and EAE is widely considered to be a relevant model for the human disease. TNFα and LT are key elements in the pathogenesis of MS and EAE (reviewed by Steinman[6]).

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TNFα and LT in EAE

Many studies indicate that TNFα activity is increased during active disease. TNFα and LT mRNA can both be detected in the CNS in acute EAE, and are made predominantly by microglia and infiltrating macrophages.[7] LT increases before the onset of clinical signs of EAE, while TNFα peaks at the height of clinical disease and during relapses.[7–9] TNFα given systemically worsens the severity and duration of EAE and can trigger relapses.[10 11] TNFα and LT are both directly toxic in culture to oligodendrocytes, cells that form the myelin sheath in the CNS.[12 13] TNFα given directly into the vitreous chamber in mice causes demyelination of the optic nerve.[14]

TNFα production by astrocytes can be induced in culture. There is higher production of TNFα in astrocytes from rodent strains which are susceptible to EAE.[15] The ability of MBP reactive T cell clones to transfer EAE correlates with their level of production of TNFα and LT.[16] Altered peptide ligands reduce the production of TNFα and Th1 cytokines, and can reverse EAE.[17 18]

Bacterial superantigens can induce relapsing attacks of paralysis in EAE, an effect blocked by antibody against TNFα.[19] TNFα delivered locally by a T cell clone carrying a TNFα encoding retrovirus construct exacerbates EAE.[20]

Reduction of TNFα activity by a number of different means abrogates disease. Treatment of mice with anti-TNFα antibody[21 22] or neutralisation of TNFα and LT activity with soluble p55 TNF receptor[23–25] blocks development of EAE. Rolipram, a selective phosphodiesterase type IV inhibitor that reduces production of TNFα and LT, reduces clinical signs of EAE.[26–28]

In summary, these studies lead to the conclusion that increased levels of TNFα activity exacerbate disease, while blockade of TNFα lessens disease in EAE models.

TNFα and LT transgenic and knockout mice

Transgenic experiments indicate that overexpression of TNFα can induce spontaneous disease. Overexpression of TNFα in the CNS of transgenic mice causes spontaneous inflammatory demyelination.[29–31] Oligodendrocyte apoptosis and myelin vacuolation is observed in these animals.[32] The effects of TNFα are prevented if the p55 TNF receptor is knocked out.[32] Overexpression of TNFα in the CNS,

even in mice lacking CD4, β-2 microglobulin, immunoglobulin μ chain, and RAG-1, is sufficient to induce demyelination.[33]

Gene knockout studies have examined the role of TNFα and LT in susceptibility to induced EAE. A complicating factor in several studies[34 35] is that disruption of these genes causes developmental defects, such as abnormal lymph nodes, altered splenic architecture, and abnormal immune function. In a more recent study of TNFα and LT knockout mice, immunodeficiency was corrected in LTα-/- mice by reconstitution with bone marrow cells.[36] Mice with TNFα knocked out, but with LTα present, had a delayed onset and shorter duration of disease. The absence of TNFα had the effect of impairing lymphocyte migration into the CNS. When LTα was knocked out, but TNFα was present, EAE developed normally. Similar results were seen in an earlier study of TNFα knockout mice.[37] The conclusion from these knockouts is that TNFα plays an important part in lymphocyte trafficking into the CNS.

Although TNFα is generally proinflammatory in EAE experiments, TNFα may be anti-inflammatory under some conditions. TNFα reduced EAE when given systemically using a recombinant vaccinia virus.[38] The effects of TNF and other cytokines vary depending on whether they are given systemically versus locally, timing of administration, and other factors. In another study of TNFα-/- knockout mice, MOG induced EAE was more severe in TNFα-/- mice than in littermate controls.[39] TNFα given systemically was protective and prevented development of EAE. Presumably the explanation is one of cytokine redundancy, with other molecules substituting functionally for TNFα in this situation. Similarly, Th1 cells are generally implicated in EAE, and Th1 clones reactive to MBP can transfer disease.[40] However in the study of Lafaille *et al*,[41] anti-MBP Th2 cells were derived from MBP specific TCR transgenic mice, and transferred into RAG-1 knockout mice. The transferred anti-MBP specific Th2 cells were able to cause EAE. IL4 was present in the lesions, but no TNFα or other Th1 cytokines were present.

Despite these exceptions, the same generalisations hold true from experiments with TNFα transgenic and knockout mice. TNF expression in transgenic mice is sufficient to cause spontaneous inflammatory demyelination, while inactivation of the TNFα gene impairs lymphocyte migration into the CNS.

TNFα and LT in MS

As in EAE, TNFα and LT protein and mRNA can be demonstrated in MS plaques.[42 43] TNFα positive cells include lymphocytes, macrophages, endothelial cells, astrocytes, and microglia.[44 45]

TNFα is present in the CSF of subjects with MS,[46-48] and the level of TNFα correlates with severity and progression of disease.[49] TNFα increases the permeability of CNS endothelial cells.[50] TNFα levels in CSF are higher in MS subjects with active disease and correlate with blood-brain barrier damage.[51] CSF mononuclear cells from MS subjects show increased TNFα mRNA levels, compared with blood mononuclear cells, or compared with cells from the CSF of control subjects.[52 53] PBLs maintained in culture from MS subjects produce more TNFα and LT than controls.[54] Disease exacerbation is correlated with higher levels of TNFα and LT mRNA in PBLs.[55-58] TNFα production after mitogen stimulation of PBLs from MS subjects is increased before exacerbations.[59]

As well as being toxic to oligodendrocytes, TNFα, and to a lesser extent LT, is mitogenic for astrocytes in culture, and may contribute to reactive gliosis found in MS.[60 61]

Like EAE, these data suggest that higher levels of TNFα correlate with increased disease activity in MS.

TNF genes and susceptibility to MS

The genes encoding TNFα and LT are embedded within the major histocompatibility complex (MHC) in the human chromosomal segment 6p21, about 250 kb centromeric to the HLA-B gene and 355 kb telomeric to C2. Multiple polymorphisms have been identified in or near the TNF genes. However case-control population based studies have failed to detect significant genetic association with MS susceptibility or progression. Results indicating distortions of the expected allelic frequencies could be attributed to the effect of linkage disequilibrium with HLA-DR2.[62]

Current treatments for MS: effects on TNFα

Several currently used treatments for MS have effects on TNFα activity. Methylprednisolone intravenously, or oral prednisone, is commonly used in the treatment of MS exacerbations. The effects of corticosteroids include a decrease in TNFα activity by inhibiting transcription.[63] There is a reduction of MMP-9 activity (gelatinase B), and increased levels of TIMP-3.[64]

Two forms of IFNß are currently used for the treatment of MS, IFNß1b (Betaseron) and IFNß1a (Avonex, Rebif). IFNß has several mechanisms of action (reviewed by Yong *et al*[65]). Pre-treatment of T cells with IFNß decreases VLA-4 integrin expression and reduces subsequent TNFα production by microglia.[66] IFNß may favour the production of anti-inflammatory cytokines,[67-71] and decrease TNFα production.[72 73] IFNß decreases TNFα receptor levels on T cells.[74] IFNß decreases MMP-2 and MMP-9 activity and reduces T cell migration into the CNS.[75 76]

Copolymer-1 (Copaxone), a random copolymer of alanine, glutamic acid, tyrosine, and lysine, is the most recent immunomodulatory drug approved for the treatment of MS. Recent studies suggest that copolymer-1 acts as an altered peptide ligand, causing a shift from a Th1 to a Th2/Th3 response, with a decrease in TNFα mRNA levels.[77]

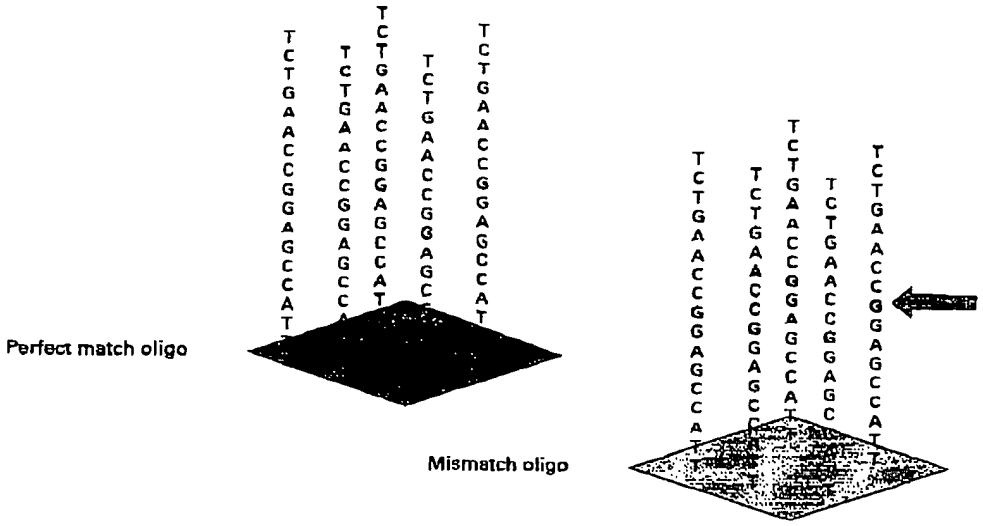

Figure 1 Chip design. A perfect match (PM) and an adjacent mismatch (MM) 25-mer DNA oligonucleotide probe pair is illustrated. The MM oligonucleotide has a single change at position 13.

Therapeutic blockade of TNFα activity in MS

A study of two rapidly progressive MS subjects treated with the anti-TNFα antibody cA2 (Remicade, Centocor) was reported by Van Osten *et al* in 1996.[78] The subjects were given two infusions of 10 mg/kg of antibody at intervals of two weeks. There was no reported clinical worsening of disease. However, an increase in the number of gadolinium enhancing lesions on magnetic resonance imaging, a rise in CSF IgG index, and an increase in the number of lymphocytes in the CSF was observed after each infusion. The magnetic resonance imaging and CSF findings were felt to indicate intrathecal immune activation. VCAM-1 was detectable in CSF post-infusion, while levels of VCAM-1 and ICAM-1 were lower in serum. TNFα production by stimulated WBCs was lower after treatment. cA2 antibody could not be detected in CSF, and was probably not able to cross the blood-brain barrier. Unfortunately, there were no monthly pre-treatment scans to estimate ongoing disease activity in this small study.

A soluble dimeric p55 TNF receptor-immunoglobulin fusion protein (sTNFR-IgG p55; lenercept, Roche) was tested in a double blind placebo controlled study of 168 mainly relapsing-remitting MS subjects.[78a] Subjects were treated with 10, 50, or 100 mg of lenercept intravenously every four weeks, for a period of up to 48 weeks. Clinical evaluations and MRI were obtained every four weeks. The exacerbation rate was increased by 2%, 68%, and 50%, over the placebo rate, in subjects treated with 10, 50 and 100 mg of lenercept respectively ($p = 0.007$). There was a dose dependent decrease in the time to first exacerbation in treated groups ($p = 0.006$). There

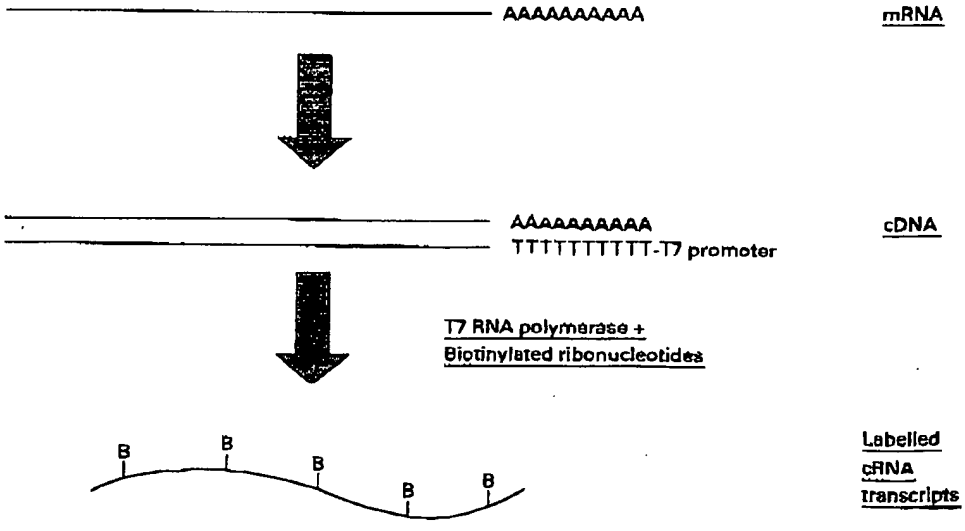

Figure 2 Sample preparation. A primer containing oligo-dT (T24) and a T7 RNA polymerase binding site is used for first strand cDNA synthesis. Double stranded cDNA is used for an in vitro transcription reaction, to make cRNA labelled with biotin-UTP and biotin-CTP.

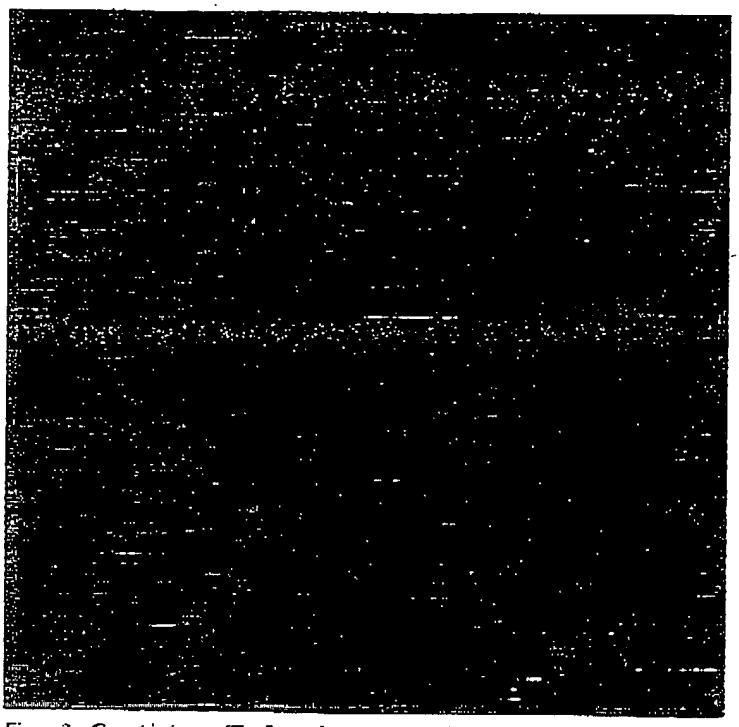

Figure 3 Genechip image. The figure shows a scanned image of a HuGeneFL chip.

was a tendency for the duration and severity of exacerbations to be increased with lenercept, although this was not statistically significant. Side effects included headache, hot flushes, nausea, dyspnoea, and abdominal pain. Antibodies to lenercept were detected in most of the treated subjects. Trough serum concentrations of lenercept were detected in only a third of patients, and not in those subjects with high anti-lenercept antibody concentrations. New occurrences of rheumatoid factor or ANA were more common in the lenercept treated group. Despite clinical worsening, there were no significant changes in MRI measurements between groups. One explanation may be the timing of scans in relation to drug dosing. MRI scans were obtained four weeks after the preceding dose, and changes may therefore have been missed. However, it is also recognised that MRI changes have poor pathological specificity in MS, and poor correlation with clinical measures of disability. Newly active MRI lesions reflect changes in blood-brain barrier permeability, which may perhaps be separate from CNS inflammation.

Pentoxyfilline, a phosphodiesterase inhibitor, reduces TNFα production and prevents EAE[79 80] and has been studied in MS. TNFα production is reduced in vitro,[81] but there is no apparent effect on disease.[82 83] Perfenidone, an experimental drug that prevents gliosis and blocks TNFα synthesis, is currently being tested in MS.

To date the results of TNFα blockade in MS have been disappointing, and seem to make disease worse, or at best have no effect. These results are unexpected and in contrast with animal models, where TNFα blockade is effective in ameliorating disease. The reasons for this difference are not clear at the present time.

Metalloproteinase inhibitors

The MMPs are a group of zinc containing proteolytic enzymes involved in the degradation and remodelling of the extracellular matrix.[84] The family consists of approximately 18 members subdivided into collagenases, gelatinases, stromelysin, and membrane-type MMPs. T cells express predominantly MMP-2 (gelatinase A) and-9 (gelatinase B), while macro-

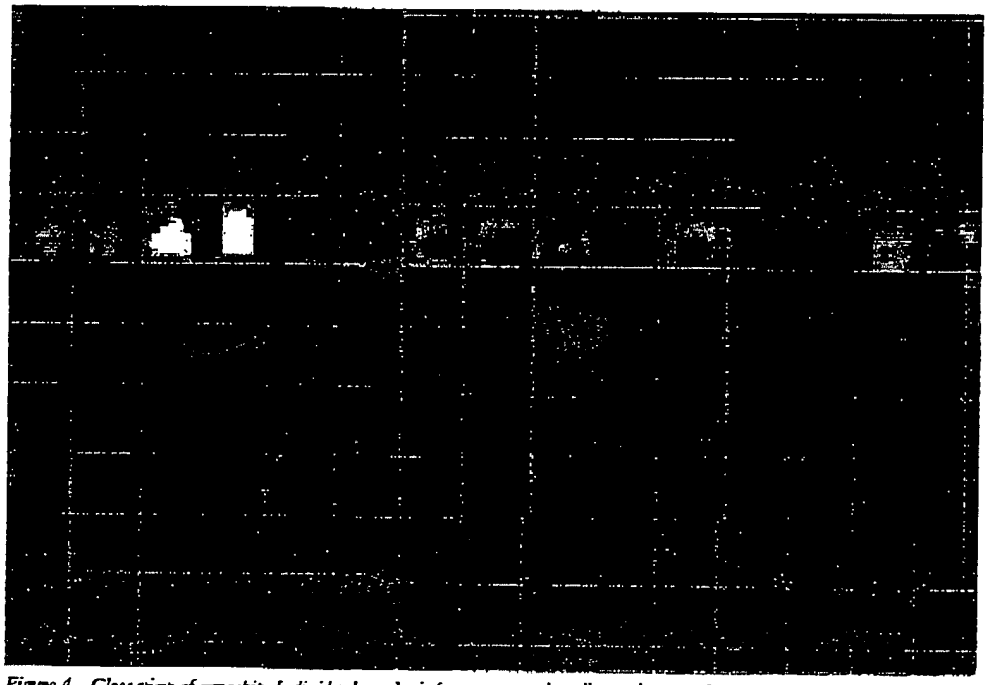

Figure 4 Close view of genechip. Individual synthesis features or probe cells can be seen. Each 24 × 24 μm probe cell is imaged by the scanner as a series of 3 μm pixels. The software aligns a grid to the image after scanning. A row of brighter PM probes can be seen, above a row of corresponding MM probes.

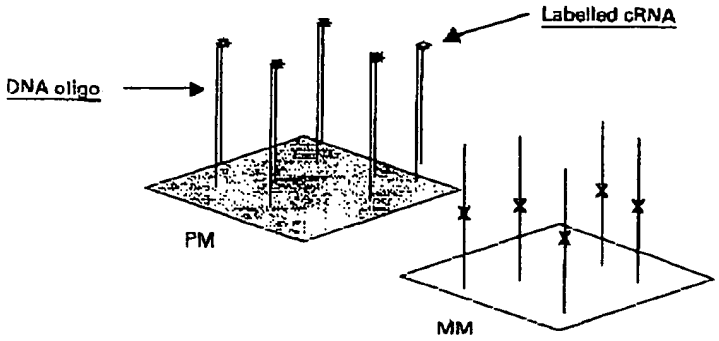

Figure 5 Hybridisation. Biotinylated cRNA, stained with streptavidin-phycoerythrin, binds preferentially to the PM oligonucleotide. Hybridisation is measured by calculation of PM-MM differences, and PM/MM ratios. The software makes a call of increased, decreased, or no change for each RNA, when comparing two chips.

phages secrete a broader range and larger amounts of MMPs,[84] including MMP-1, -2, -3, -7 (matrilysin), -9 and -12. Neurons, astrocytes, and oligodendrocytes also express MMPs.[85–89] The activity of MMPs is tightly regulated at the level of transcription, by proenzyme activation, and by the activity of tissue inhibitors of metalloproteinases (TIMPs). Gene transcription can be induced by growth factors, inflammatory cytokines, and via cell-ECM or cell-cell interactions. MMPs are initially produced as inactive pro-MMPs, containing a zinc atom bound to a cysteine residue in the catalytic domain. Activating factors disrupt the zinc-cysteine interaction and expose the catalytic site. After activation, MMPs are regulated by the formation of complexes with one of the four TIMPs. MMP activity is tightly controlled by these means, but excess MMP production and activation may be an important factor in autoimmune diseases, including MS.

Table 1 TNF related genes represented on HuGeneFL array

Accession	*Entrez definition*
HG4683-HT5108	Tumour necrosis factor receptor 2 associated protein Traf3
M16441	Lymphotoxin gene extracted from human tumour necrosis factor and lymphotoxin genes, complete CDs
M31165	Human tumour necrosis factor inducible (TSG-6) mRNA fragment, adhesion receptorCD44 putative CDS
M31166	Human tumour necrosis factor inducible (TSG-14) mRNA, complete CDs
U 12595	Human tumour necrosis factor type 1 receptor associated protein (TRAP1) mRNA, partial CDs
M32315	Human tumour necrosis factor receptor mRNA, complete CDs
M59465	Human tumour necrosis factor alpha inducible protein A20 mRNA, complete CDs
U77396	Human TNF alpha inducible responsive element mRNA, complete CDs
U86755	Human TNF alpha converting enzyme mRNA, complete CDs
U69611	Human TNF alpha converting enzyme mRNA, complete CDs
U78798	Human TNF receptor associated factor 6 (TRAF6) mRNA, complete CDs.
D78151	Human mRNA for 26S proteasome subunit p97, complete CDs
D38047	Human mRNA for 26S proteasome subunit p31, complete CDs
X02910	Human gene for tumour necrosis factor (TNF alpha)
V01512	Human cellular oncogene c-fos (complete sequence)
J04111	Human c-jun proto oncogene (JUN), complete CDs, clone hCJ-1
M58286	Homo sapiens tumour necrosis factor receptor mRNA, complete CDs
L41690	Homo sapiens TNF receptor-1 associated protein (TRADD) mRNA, 3' end of CDs
U69108	Homo sapiens TNF receptor associated factor 5 mRNA, partial CDs
Z22951	Homo sapiens gene encoding p65 subunit of transcription factor NF-kappaB
L04270	Homo sapiens (clone CD18) tumour necrosis factor receptor 2 related protein mRNA, complete CDs
Y10256	Homo sapiens mRNA for serine/threonine protein kinase, NIK

The HuGeneFL probe array is a high density, single chip array with over 6000 genes represented. Full length genes were selected from UniGene, GenBank, and TIGR databases by Affymetrix. TNFα, LT, TNF receptors, and a number of genes involved in TNFα signalling pathways are represented on the array.

MMPs are needed to facilitate T cell penetration through the basement lamina of the vascular endothelium into the CNS. Increased MMP expression can be demonstrated in the CNS of subjects with MS[90–93] and in animals with EAE.[96 97] MMP inhibitors block degradation of the extracellular matrix and reduce entry of T cells into the CNS. One mechanism of action of IFNß in MS is the inhibition of MMP-9 activity of T lymphocytes.[73 76]

TNFα is initially produced as a 26 kDa membrane anchored protein and is converted to the mature, secreted 17 kDa protein by TNFα converting enzyme (TACE). MMP inhibitors prevent the conversion of TNFα into an active form. In addition, MMPs contribute directly to the degradation of myelin proteins, and MMP inhibitors may block this activity.

MMP inhibitors can block induction of EAE.[98–102] A small trial in MS subjects of a combination of D-penicillamine and metacycline, which inhibit MMP-9 and t-PA, was reported. Ten patients with secondary progressive MS were treated over a period of one year. There was no improvement in Extended Disability Status Scale (EDSS) scores at one year, and there were problems with toxicity.[103] Several MMP inhibitors are currently being tested in clinical trials in MS, but there are no published data at the present time.

Genomics and microarray technology

Current estimates suggest that there are approximately 100 000 genes in the human genome, and about one half of these have been partially or completely sequenced. Determination of which of the 100 000 genes are expressed is a useful initial step in understanding a disease process.

Microarray technology allows a large scale readout of gene expression. Several different types of microarrays are available, based on cDNAs, PCR products, or oligonucleotides immobilised on a solid support. The Affymetrix Genechip is a high density oligonucleotide array synthesised directly onto glass slides by a combination of photolithography and light activated chemistry.[104 105] The expression arrays contain as many as 400 000 24 × 24 µm synthesis features. Each synthesis feature or probe cell contains approximately 10^7 copies of a specific 25-mer DNA oligonucleotide sequence. The synthesis features are organised in pairs, consisting of a perfect match (PM) oligonucleotide and a mismatch oligonucleotide (MM) immediately below (fig 1). The MM oligonucleotide is identical except for a single base change at the middle position, and is used as a control for hybridisation specificity. Each gene on the array is represented by a series of 20 probe pairs, which span the sequence.

Samples are prepared for hybridisation to the array, first by isolation of polyA+ mRNA. Double stranded cDNA is then made, and the cDNA is used as a template to produce biotinylated cRNA (fig 2). The labelling procedure amplifies the mRNA population by about 150-fold. The cRNA is fragmented and hybridised to the array, forming hybrids

between the biotinylated cRNA and the DNA oligonucleotides on the chip. The array is washed, stained with streptavidin-phycoerythrin, and scanned with a confocal laser microscope. A scanned image of a genechip is shown in figure 3, and a close up view in figure 4.

The average fluorescence intensity is calculated for each probe cell. The presence or absence of a particular RNA is determined from the hybridisation pattern, using PM and MM differences and ratios (fig 5). The signal is proportional to the amount of bound, labelled cRNA. The relative concentrations of different RNAs in a population can be estimated from the signal intensity. A single sample is applied to each array, and the software compares arrays.

Using this system, we have analysed several rodent EAE models, and human MS samples (unpublished data), to look for differentially expressed genes. The aim is to identify novel targets for drug development. A number of TNF related genes are represented on the arrays (table 1). Many genes known to be involved in demyelination are differentially expressed, along with a number of less well characterised genes. The data are complex, but provide a more global view of gene expression. A difficulty with this type of data is that it does not give functional information regarding the identified genes. Functional experiments such as gene inactivation, cellular localisation, and other types of studies are needed. Cluster analysis may provide functional clues by grouping together genes whose expression is co-regulated.[106] The size of the data sets also presents a challenge.

Genomics has been likened to the biological equivalent of the chemical periodic table, but with 100 000 gene elements and an information space with more than two dimensions.[107] The microarray approach will hopefully provide a more comprehensive view of TNF related pathways involved in demyelinating and other diseases.

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